PR

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ERRATA

CAREY, MARY J., CONWAY, E. J. & KERNAN, R. P. (1959).
J. Physiol. **148**, 51–82.

p. 68 and p. 70: the curves shown in Fig. 6 should have appeared as Fig. 7, and vice versa.

p. 75, l. 14: *for* '140 mM-Na' *read* '104 mM-Na'.

p. 81, l. 2: *for* '10·9% *read* '109%'.

J. Physiol. (1960), **150**, *pp.* 1–33
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SUGGESTIONS TO AUTHORS

Papers should consist primarily in the presentation of the authors' own original experimental work. Theoretical discussions alone, or of disproportionate length, are not in general accepted. Papers should be as brief as possible in order to reduce editorial work, limit the cost of printing, lighten the burden of the reader, and accelerate publication. Excessive brevity is more easily mended than prolixity.

The reader needs to know not only the results and conclusions of the research but also the degree of reliance that can be placed on them. The author should give sufficient evidence to indicate the precision of methods and adequacy of controls.

It is important to remember that a reader is often influenced by literary style, and the author in his own interest should take pains to convey his meaning as simply and accurately as possible. Care should be taken in the choice of words and their placing in the sentence, as well as in the sequence and linkage of sentences. The first person should not be used repeatedly, but its use is sometimes permissible if an awkward periphrasis is thereby avoided. The term 'we' should not be used by a sole author. Sir Ernest Gowers's *Plain Words* and *ABC of Plain Words*, H. W. Fowler's *Modern English Usage* and Sir Arthur Quiller-Couch's *The Art of Writing* (especially the chapter on 'jargon') may be found helpful. Spellings should follow the *Oxford English Dictionary*. Roman and italic words and contractions both English and foreign are to be found in Collins's *Authors' and Printers' Dictionary*.

Before submitting a manuscript for publication, it is nearly always valuable to ask someone not acquainted in detail with the work to criticize it. The interval between receipt of a paper and its publication will be shortened by careful attention to the instructions below.

ARRANGEMENT

Papers in recent numbers of *The Journal of Physiology* should be studied both for the general manner of presentation and for detail. Except in special circumstances the paper should be subdivided into:

(1) Introduction,
(2) Methods,
(3) Results,
(4) Discussion and inferences,
(5) Summary,
(6) Acknowledgements,
(7) References.

(1) The introduction should make clear the object of the research. Reference

to previous work is desirable only if it has a direct bearing on the subject of the paper; an extensive historical review is seldom necessary.

(2) Methods need description once only. Sufficient detail should be given to allow the work to be repeated by others.

(3) As a rule the results are best given in words. Large numerical tables from which the reader himself is expected to draw conclusions should be avoided. One or a few graphs may simplify the reader's task. Experiments which are essentially similar to the leading ones in nature and result should not be described in detail, though it is important to mention that they have been done. The precision to be attached to numerical values should be given when possible. Theory and deduction must be clearly distinguished from what has actually been observed, and, if possible, should not be elaborated in this section.

(4) A discussion follows the statement of results and is kept separate from it. In this section clarity of expression is particularly important, and the assumptions made in reaching deductions from the experimental results must be stated. The discussion should not merely recapitulate the results.

(5) The summary should be arranged in short numbered paragraphs and should not be longer than one-twentieth of the text. It must not contain anything that is not included in the body of the paper. The summary may be used as it stands by contributors to abstracting journals. It should therefore contain a succinct account of the problem in hand, the method and materials, results and conclusions.

(6) Acknowledgements, which will be printed in small type, should be reduced to the minimum compatible with the requirements of courtesy.

(7) The paper is completed by an alphabetical list of the papers and books which have been referred to in the text. Details of the form in which the references should be given will be found on pp. 7–9.

(8) An abstract suitable for inclusion in *International Abstracts of Biological Sciences* is also required. Instructions for its preparation are given on p. i of each issue of the *Journal*.

PREPARATION OF MANUSCRIPT

Papers sent for publication must be typewritten, with double spacing and wide left-hand margins, on sheets of uniform size, e.g. all foolscap or all quarto. The top copy of the typescript should be sent for the printer. Papers should be carefully corrected and as nearly ready for press as possible before submission. Insertions should be few and legible. Papers and illustrations must whenever possible be packed flat.

Title

The title is to be typed on a separate sheet. The name of the author follows, together with any necessary description, e.g. *Beit Memorial Research Fellow*.

When there are several authors their names will be printed in alphabetical order. An authoress should give one Christian name to indicate her sex. The name of the laboratory and its address complete the title. Any instructions with regard to dispatch of proofs should also be typed on the title sheet.

A short title also is needed, to be printed at the head of the right-hand pages in the *Journal*. Authors are asked to supply the short title; it must not exceed 43 letters + spaces, and should be less if possible.

The Editors will not accept a series of papers with the same main title, e.g 'Studies in..., Part I (II, III,...)'. Each paper must have its own title.

Instructions to the printer

The section headed 'Methods' will normally be printed in small type. This section, and any other matter to be so treated, is to be marked by a vertical line in the left-hand margin. Words (usually headings) to be printed in *italics* are indicated by one underline. Two underlines indicate SMALL CAPITALS, three indicate LARGE CAPITALS. The author should mark the desired positions of tables and figures, and should do so by writing instructions enclosed within lines and brackets, e.g.

(Table 3 near here) or (Fig. 4 near here)

Headings in the text

Six grades of headings and subheadings may be used in *The Journal of Physiology*, but in general they are restricted to four. The possible grades are as follows:

1.	PART I	(Capitals)
2.	RESULTS	(Small capitals)
3.	The normal intestine	(l.c. roman)
4.	*The action of drugs*	(Italics, centre)
5.	*Histamine*	(Italics, full out)
6.	*Peristalsis*. This type of movement...	(Italics, indent)

The usual headings required are 2, 4 and 6. If further subdivision is needed within one of the main sections, No. 5 should be used as well. Recent numbers of the *Journal* should be consulted for further examples of the customary use of headings. Note that no full stops are required after headings except those in grade 6.

Tables

Tables are expensive to print and should be as few as possible. They should be typed double-spaced on separate numbered sheets and referred to in the text by

arabic numerals, e.g. Table 3. The expression 'the following table' should be avoided. Each table should have its own self-explanatory heading, which is generally printed in roman type and should therefore not be underlined. The same data should not normally be presented in both tabular and graphical

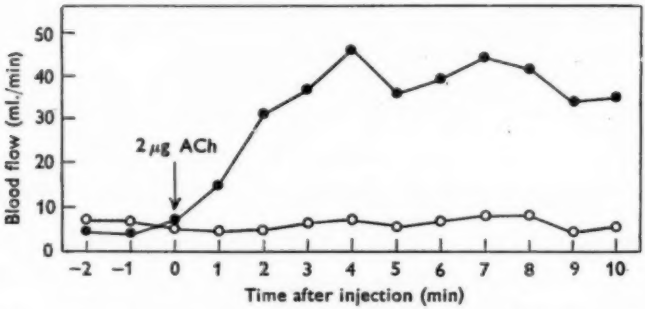

Fig. 1. A specimen figure as it would appear in *The Journal of Physiology*. Figs. 2 and 3 show how the original should be drawn to produce this result. The lettering has been inserted by the printer.

This figure also shows the customary manner of indicating the units and the scales of the axes.

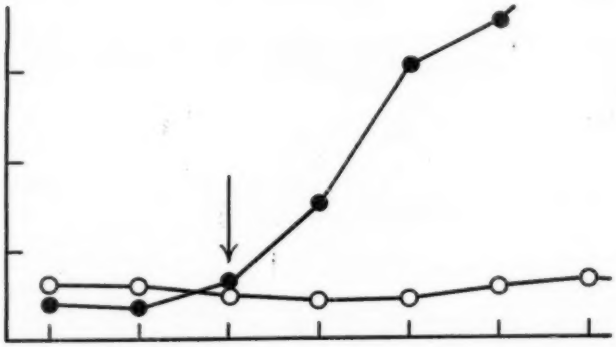

Fig. 2. Part of Fig. 1 as it should be drawn by the author for reduction to one-half size. Lettering would be in pencil.

forms. Tables should be so arranged that they can be printed without dividing lines: 'ditto' signs are not used. In preparing tables the list of standard abbreviations should be consulted. The units in which the results are expressed, e.g. g/100 ml., should be given in brackets at the top of each column, and not repeated on each line of the table. Long descriptive headings to columns should be avoided.

Illustrations

Illustrations are reproduced by photographic engravers and should therefore be separable from the text. Reference to them in the text should be given as follows: Pl. 3; Pl. 3, fig. 2; Fig. 3; Text-fig. 3. The last form is used only when the paper contains both figures in the text and plates.

Each illustration should bear on the back the proper reference number and the author's name: if there is any chance of confusion, e.g. when two papers are submitted together, the title of the paper should be added.

Illustrations from line drawings or tracings are often clearer and always less costly than half-tones from photographs. Line drawings in Indian ink on Bristol board are excellent for reproduction. They should be about twice as large as their intended final size, and should be presented separately on sheets *not larger than foolscap size* (21 × 32 cm; 8¼ × 13 in.). In the original, the lines must be thick enough to allow for reduction, as shown in Figs. 1–3. Lettering on

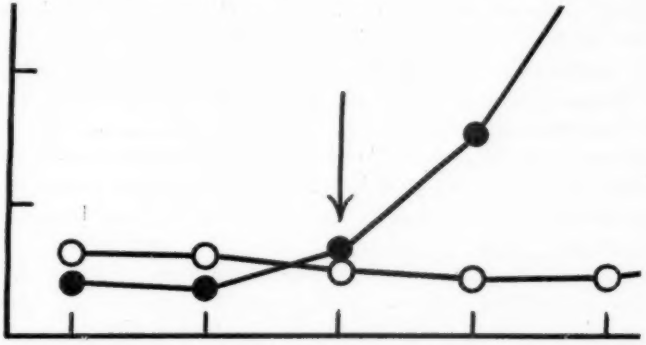

Fig. 3. Part of Fig. 1 as it should be drawn by the author for reduction to one-third size. Lettering would be in pencil.

drawings should be in pencil so that expert draughtsmen can insert suitable letters. The exact position of lettering should be indicated, and care must be taken that the symbols agree with those used in the text. Illustrations cannot easily be altered in proof stage. Graphs should be drawn on plain paper, tracing paper, Bristol board, or blue-lined paper; paper with lines printed in any colour other than blue should not be used. Symbols which are to appear in the legend should be chosen from the following, which are available as type:

× + ● ○ ⊙ ⊕ ⊗ ◑ ◐ ■ □ △ ▲ ▽ ▼ ◇ ◆

Kymograph records should include appropriate scales as far as possible. Large black areas which are not needed should be cut away. Letters and numbers on kymograph records should be large enough to allow for photographic reduction. They are often, however, best left for the engraver to insert.

Half-tone reproductions lose some of the contrast and definition of the original. Hence the original should be excellent in these respects or, if not, it should be strengthened by an intensifier or copied by means of process plates. If a record or photograph is retouched, this must be stated in the legend.

Difficulties may occur in reproduction

(*a*) when graphite pencil has been used in retouching; the shiny surface may reflect light so that what should be 'black' is reproduced 'white';

(*b*) with ink-written (e.g. manometer) tracings on coloured paper;

(*c*) where the actual trace in oscilloscope or similar records is surrounded by a 'penumbra', which in the print may obscure the trace itself. Shading caused by scatter of light should be removed, e.g. by covering with Chinese white, by gas-light paper reproduction, etc.

The half-tone process will sometimes afford a way round this trouble, though at extra expense and usually with some sacrifice of detail.

Legends or captions should be typed separately from the illustrations. It is of assistance to the printer if they are each typed double-spaced at the foot of a separate standard-sized sheet and appropriately numbered. Magnifications, especially in photomicrographs, should be indicated by a scale on the photograph itself, since a magnification stated in the legend will become inappropriate if the photograph is reduced.

Figures should be comprehensible without reference to the text. If the same arrangement or symbols are used in several figures it may, however, be permissible to describe them in detail once only, and subsequently to refer to the original legend.

The original drawings should always be sent for making the blocks, but it is very helpful if authors also provide photographic copies of the figures with lettering added in ink and with duplicate legends attached, both for use by editors and referees and to indicate the position of lettering to be inserted by the Press. Photographic copies *must* be provided when the originals exceed foolscap size.

Quotations. In quotations use single quotes, i.e. ' ' and not " ".

Footnotes. Footnotes are expensive and tiresome; they are best avoided.

Proofs

Proofs are sent to authors in order that they may correct mistakes in printing and not that they may add new material. If they do add new material there is risk of delay in publication, and increased printing charges to the Society are inevitable. Excessive alteration may have to be disallowed or charged to the author. It is therefore important that the original typescript should be as free as possible from errors. If, however, alteration in the middle of a paragraph is unavoidable, the insertion should be arranged to fit neatly without upsetting

neighbouring lines of unoffending type. Should some amplification or correction become necessary when the paper has already reached proof stage, it is often better to add a note at the end of the paper, headed 'Note added in proof', rather than to make extensive insertions and alterations in the text.

Proofs should always be corrected and returned promptly. Unless otherwise directed, proofs are sent to the author whose name is first alphabetically, at the address from which the paper originates. If the author wishes the proofs to be sent to any other person or any other address instructions should be given. When postal delays are likely to occur, authors residing in other countries may nominate someone in Great Britain who would be willing to correct the proofs. Papers from such contributors should be accompanied by a statement of the number of reprints required.

Authors are recommended to read R. W. Chapman's essay 'Author and Printer' in Collins's *Authors' and Printers' Dictionary*. For signs used in proof correction, see pp. 18–19. 'O.K.' may be ambiguous.

REFERENCES

In the text, references to other writings are made by giving in brackets the name of the author and the year of publication, e.g. (Hill, 1938), except when the author's name is part of the sentence, e.g. 'Hill (1938) showed that...'. When a paper written by two authors is quoted, both names are given, the ampersand (&) being used in place of 'and', e.g. Fletcher & Hopkins (1907). If there are more than two authors all names should be given when cited for the first time, and thereafter the first name only, adding '*et al.*' When several papers are referred to in the same bracket, those by the same author should be separated by commas and those by different authors should be separated by semicolons, e.g. (Fletcher & Hopkins, 1907; Hill, 1938, 1949*a*, *b*). Unpublished material may be referred to sparingly in the text by giving the author's name followed by 'unpublished' or 'personal communication', and should not appear in the list of references. Authors are requested to verify that the wording of references to unpublished work and of acknowledgements of help or criticism is approved by the persons concerned.

References should be brought together at the end of the paper, double-spaced and in alphabetical order, each giving:

Name(s), followed by initial(s), of author(s).

Year of publication in brackets. When the publication of a volume is spread over more than one year, give, if possible, the publication year of the article in question. If several papers by the same authors in one year are cited, *a*, *b*, *c*, etc. are placed after the year of publication.

Full title of article.

Journal's title, abbreviated in accordance with the list on pp. 20–33 and underlined to indicate italics.

Volume number in arabic numerals underlined with a wavy line to indicate heavy type, without prefix 'vol.'

The numbers of the first and last pages in arabic numerals, without prefix 'p'. Volume and page numbers should be replaced by the phrase 'in the Press' in the case of a paper accepted for publication by a journal but not yet printed.

Thus, references should appear in the typescript as shown in Fig. 4, and will be printed in the following form:

FLETCHER, W. M. & HOPKINS, F. G. (1907). Lactic acid in amphibian muscle. *J. Physiol.* **35**, 247–309.

HILL, A. V. (1938). The heat of shortening and the dynamic constants of muscle. *Proc. Roy. Soc.* B, **126**, 136–195.

HILL, A. V. (1949*a*). The absence of lengthening during relaxation in a completely unloaded muscle. *J. Physiol.* **109**, 8–9 *P*.

HILL, A. V. (1949*b*). The energetics of relaxation in a muscle twitch. *Proc. Roy. Soc.* B, **136**, 211–219.

REFERENCES

Fletcher, W.M. & Hopkins, F.G. (1907). Lactic acid in amphibian muscle. J. Physiol. 35, 247-309.

Hill, A.V. (1938). The heat of shortening and the dynamic constants of muscle. Proc. Roy. Soc. B, 126, 136-195.

Hill, A.V. (1949a). The absence of lengthening during relaxation in a completely unloaded muscle. J. Physiol. 109, 8-9P.

Hill, A.V. (1949b). The energetics of relaxation in a muscle twitch. Proc. Roy. Soc. B, 136, 211-219.

Fig. 4. A specimen list of references, correctly typed for submission to *The Journal of Physiology*. The same list is printed on this page as it would appear in the *Journal*.

Titles of books, and the abbreviated names of periodicals, are underlined singly to indicate italics. Volume numbers are given a wavy under-line to indicate heavy type. Authors' names will be printed in capitals and small capitals without special indication on the type-script.

When reference is made to a book, the title should be underlined; the edition

(ed.), volume number (vol.) and page (p. or pp.) should be given, followed by the town of origin and the publisher, thus:

STARLING, E. H. (1915). *Principles of Human Physiology*, 2nd ed. p. 184. London: Churchill.

If the book is by more than one author, reference should be made to that part and that author concerned, thus:

GODDARD, D. R. (1945). The respiration of cells and tissues. In HÖBER, R., *Physical Chemistry of Cells and Tissues*. Philadelphia: Blakiston; London: Churchill.

or

CRANE, E. E. (1950). Bioelectric potentials, their maintenance and function. In *Progress in Biophysics*, vol. 1, ed. BUTLER, J. A. V. & RANDALL, J. T. London: Butterworth-Springer.

When particular parts, fascicles or numbers, with roman numerals or other system not here used, need reference, ordinary abbreviations should be used, thus:

HERMANN, L. (1879). *Handbuch der Physiologie*, Band 2, Theil 1, p. 171. Leipzig: Vogel.

On the rare occasions when it is necessary to refer to an author at second hand, the reference should appear in the following form:

HERING, E. (1915). Das Purkinjesche Phänomen im zentralen Bezirke des Sehfeldes. *v. Graefes Arch. Ophthal.* **90**, 1. Cited by HECHT, S. in *Physiol. Rev.* 1937, **17**, 239–290.

or

McGRADY, E., JR. (1944). Evolution and significance of germ layers. *J. Tenn. Acad. Sci.* **19**, 240–251. Cited in *Brit. Abstr.* A III, 1945, 150.

In a reference to a publication which covers a specific period but which may be issued in a later year, the year of issue should follow the author's name, and the year covered by the volume should be placed after the title of the journal:

COLE, L. B. (1927). The use of Lugol's iodine in exophthalmic goitre. *St Thom. Hosp. Rep.* 1925, **49**, 255–266.

Attention should be paid to the customary punctuation in the list of references.

MATHEMATICAL NOTATION

The rules laid down for mathematical notation and numerals in *Proceedings of the Royal Society*, A, 1909, **82**, 14–17, should be followed.

The symbols conventionally used to denote particular quantities are given in the Report of the Symbols Committee of the Royal Society, 1951. Some of the more commonly used symbols are listed on p. 14.

When letters of the Latin alphabet are used as symbols for physical quantities or variables, they are printed in italic type, e.g. g (acceleration due to gravity), V (potential difference). Roman type is used for letters denoting (1) names of units, e.g. g (gram), V (volt); (2) purely mathematical constants and operations, e.g. e (base of natural logarithms), $\mathrm{d}/\mathrm{d}x$ (differential coefficient with respect to x); (3) chemical elements.

A numeral should not, in general, be used to begin a sentence, but numerals may be used in the text as abbreviations for specific quantities. It is, however, permissible to use numerals at the beginning of a sentence when they refer to a number of units which are themselves expressed in an abbreviated form, e.g. '10 ml.', but 'Twenty animals'; not 'Ten ml.', nor '20 animals'. Numbers above 100 are preferably given as numerals in all circumstances, but it is advisable not to begin a sentence in this way.

Fractions and ratios should be written 1/100, not $\frac{1}{100}$. Such common fractions as $\frac{1}{2}$, $\frac{1}{4}$, etc. are, however, permissible. As far as possible, the decimal system should be used to indicate fractions. The decimal point must always be preceded by a figure, 0 being prefixed if necessary, e.g. 0·1 must be written, never ·1. In a column of numbers containing decimal fractions the decimal points should always be arranged in the same vertical line. The metric equivalent should be added whenever units of another system (e.g. feet, pounds) are employed.

The solidus (/) is normally used in place of 'per' in expressing units, e.g. cm/sec, not cm per sec, for a velocity. Ambiguity may result if more than one solidus is used, as in V/cm/sec for a rate of change of voltage gradient; this can be interpreted as volts per (cm/sec) instead of (V/cm) per second. It would be correct to write V/cm.sec, or negative indices may be employed, e.g. $V.cm^{-1}.sec^{-1}$. The two conventions should not be mixed ($V.cm^{-1}$/sec). Occasionally it is better to retain 'per'; e.g. '(V/cm) per second'. Abbreviations are not normally used after 'per'.

When a mean is given together with a measure of variation, it is essential to indicate what measure is intended. Thus $7{\cdot}1 \pm 0{\cdot}23$ may be taken to imply either that 0·23 is the standard error of the estimated mean (S.E. of mean), or that it is the standard deviation of individual observations about the mean (S.D. of an observation).

CHEMICAL NOMENCLATURE

In general, the conventions in chemical nomenclature adopted by the Chemical Society and the Biochemical Society should be followed. These are described in *Proc. Chem. Soc.*, 1959, 4–5, and in *Biochem. J.*, 1957, **66**, 1–16. New abbreviations should be coined only for unwieldy names, and should not be used at all unless those names occur frequently. All abbreviations must be defined at first mention, and must be avoided in the Summary.

(1) *Chemical formulae* should be written as far as possible on a single horizontal line. With inorganic substances, formulae may be used, at the discretion of the editors, wherever their interpretation is clear. With salts, it must be stated whether or not anhydrous material is used, e.g. anhyd. $CuSO_4$, or which of the different crystalline forms is indicated, e.g. $CuSO_4.5H_2O$, or $CuSO_4.H_2O$.

(2) *Solutions* of common acids, bases and salts are preferably defined in

terms of normality (N) or molarity (M), e.g. N-HCl; 0·1 M-NaH_2PO_4. The term '%' must be used in its correct sense, i.e. g/100 g of solution. 10% HCl means 10 g of hydrogen chloride in 100 g of aqueous solution, and should never be used to indicate a ten times dilution of laboratory concentrated hydrochloric acid. For 'per cent by volume', i.e. ml./100 ml., the term '% (v/v)' may be employed. To indicate that a given weight of substance is contained in 100 ml. of solution, the expression '*x* g/100 ml.' is preferred to '*x* g % (w/v)'.

(3) Such terms as acid sodium phosphate should be avoided. Salts of acids containing more than one replaceable hydrogen atom should be described by naming the actual cations, e.g. sodium dihydrogen phosphate and disodium hydrogen phosphate.

(4) The termination *ine*, and not *in*, should be reserved for bases, e.g. adrenaline, choline, atropine, etc. The termination *in* is used for certain neutral substances, e.g. dextrin, pepsin, albumin, etc.

(5) Most of the amino acids follow rule (4): e.g. leucine, glycine, thyroxine, etc. Tryptophan does not have a final *e*, since *ane* is the termination reserved for saturated hydrocarbons, e.g. methane.

(6) All alcohols should have names ending in *ol* not *ite* or *ine*, e.g. glycerol (when used as a chemical term), mannitol, etc. Compounds which are not alcohols, but which are commonly given names ending in *ol*, should be spelt with a terminal *e*, e.g. indole, iminazole, pyrrole, etc. Alcohols should be given their specific names, e.g. methanol, ethanol, etc.

(7) Fatty substances are lipids (adjective lipoid).

(8) The atomic weight of radioactive and other isotopes is indicated by a superscript on the left of the symbol denoting the element, e.g. ^{32}P.

(9) *Nomenclature of drugs.* The names used for drugs should be the English (*not* Latin) titles of the current *British Pharmacopoeia*, or its *Addenda*. For substances not mentioned in these books the names given in the *British Pharmaceutical Codex* should be used. For recently introduced drugs 'Approved Names', lists of which are published from time to time by the General Medical Council, should be employed. Where only a proprietary name is available, the manufacturer's name and the actual chemical composition of the drug should be given in brackets after the first mention of such name.

(10) A dose is singular: '5 ml. H_2O was added'. Normally the dose follows the name of the substance: 'atropine 20 μg'. When the solidus is used the name must be written on the correct side: 'atropine 20 μg/kg' (or '20 μg atropine/kg'), NOT '20 μg/kg atropine'.

(11) *Ringer's solution and its variants.* The composition of the particular solution used should be given. Such terms as 'normal saline' and 'saline' should be avoided as a first reference.

BIOLOGICAL NOMENCLATURE

When the true form of Latin names of animals or plants is used, it should begin with a capital letter. When used as an adjective, or in any other form, the word should begin with a lower-case letter, e.g. Protozoa, but protozoan; Mammalia, but mammals and mammalian.

Generic and specific names should be underlined (i.e. in italics). The specific epithet or trivial name of an animal should begin with a lower-case letter.

Names of muscles, bones, etc. should be in roman type (i.e. not italics). Adrenal is preferable to suprarenal. Care should be taken in the use of such words as dorsal, ventral, caudal, proximal, distal, anterior and posterior.

GENERAL

Avoid ambiguous multiple adjectives. 'High climbing rate' might mean (*a*) 'high rate of climbing' or (*b*) 'rate of high climbing'; the expanded phrase, using 'of', is preferable.

Be careful when using such phrases as '$2\frac{1}{2}$ times increase' to make it clear whether the result is $2\frac{1}{2}$ or $3\frac{1}{2}$ times the original. '-fold' is somewhat archaic; it should not be used with numerals or decimals. It is permissible with small numbers, e.g. 'fourfold' (no hyphen; 'twofold' generally means 'of two kinds'); with large numbers, it tends to suggest 'a round number' rather than precision.

Words and meanings shown as 'rare' or 'obsolete' should not be used. New forms should not be invented (especially by mixing Greek and Latin) when established words express the meaning. Nouns such as 'synapse', 'lesion', should not be used as verbs. 'While' should not be used of events not simultaneous. 'to section' = to divide into several sections, as with a microtome. 'data' (pl.) does not mean 'results'; 'localize' does NOT mean 'locate'; mμ = 'millimicron', nano (n) = 10^{-9}. M means 'molar' NOT 'mole'; M/l. is incorrect.

PHOTOMETRIC TERMS AND UNITS

For exact definitions of the terms authors may consult Pamphlet 233 (1953) *Glossary of terms used in illumination and photometry* issued by the British Standards Institution. For physiological aspects of photometry Y. le Grand's *Light, Colour and Vision* (Chapman & Hall, 1957) will be found very helpful.

The modern trend in photometry is to avoid an enormous diversity of units, and usually to employ the candela or the lumen, combined with units of length.

Unit of luminous intensity — Candela (cd)

This replaces the old standard candle.

Unit of luminous flux — Lumen (lm)

This is the flux emitted in unit solid angle of 1 steradian by a point source having a uniform intensity of 1 cd.

Unit of illumination — lm/m^2

This can be specified as the number of lumens falling on unit area of a surface. Conversion table:

1 metre-candle = 1 lm/m^2
1 lux = 1 lm/m^2
1 phot = 10,000 lm/m^2
1 foot-candle = 10·76 lm/m^2

Unit of luminance — cd/m^2

The luminance of a surface which obeys Lambert's Law and emits 1 lm from every square metre is $(1/\pi)$ cd/m^2. Conversion table:

1 unit = 1 cd/m^2
1 candle/sq. ft. = 10·76 cd/m^2
1 millilambert = 3·18 cd/m^2
1 foot-lambert = 3·43 cd/m^2
1 equivalent foot-candle = 3·43 cd/m^2

Unit of retinal illumination in man — Troland (td)

This is the retinal illumination when a luminance of 1 cd/m^2 is seen through a pupil of area 1 mm^2. For light of wave-length 555 mμ, taking transmission losses into account:

1 td = 0·002 lm/m^2
= 10^7 quanta.sec^{-1}.mm^{-2}
approximately

Retinal illumination in animals should be expressed in lm/m^2 for white light, or in quanta.sec^{-1}.mm^{-2} at a specified wave-length.

SOME RECOMMENDED ABBREVIATIONS AND SYMBOLS

In abbreviations for units no difference is made between singular and plural, e.g. 1 min and 40 min or 24 hr, except in cases noted below. Symbols denoting units are printed without a full stop except for British units and cases where ambiguity might result, e.g. l. (litre), distinguishing from numeral 1.

About	*ca.* (not *c.*)
Absolute	abs.
Acetylcholine	ACh
Adenosinediphosphoric acid	ADP
Adenosinetriphosphoric acid	ATP
Adrenocorticotrophic hormone	ACTH
Ampere	A
Analytical standard of purity	A.R.
Ångström unit	Å
Anhydrous	anhyd.
Ante-meridiem	a.m.
Approximately	approx.
Atmosphere (pressure)	atm
Atomic weight	at.wt.
Basal metabolic rate	B.M.R.
Bivalent, trivalent, etc., e.g. bivalent iron	Fe^{2+}
Blood pressure	B.P.
Boiling-point	b.p.
British Pharmacopoeia	B.P. (with date)
British Thermal Unit	B.TH.U.
Calculated (in table headings)	calc.
Calorie (gram calorie)	cal
Calorie, large (kilocalorie)	kcal
Candela	cd
Centimetre, square centimetre	cm, cm^2
Centimetre gram second	c.g.s.
Centimetres per second	cm/sec
Central nervous system	C.N.S.
Cerebrospinal fluid	c.s.f.
Change per 10° C rise	Q_{10}
Coefficient	coeff.
Concentrated	conc.
Concentration	concn.
Constant	const.
Corrected	corr.
Coulomb	C
Crystalline, crystallized	cryst.
Cubic	cu.
but cubic centimetre	c.c. (ml. preferred)
cubic metre	m^3
Curie	c
Current density	c.d.
Cycles per second	c/s
Decibel	db
Degrees absolute	°K
Degrees centigrade	°C
Degrees Fahrenheit	°F
Dextro- (optical rotation)	(+)-
Dextro- (configuration)	D-
3:4-dihydroxyphenylalanine	DOPA
Di*iso*propylfluorophosphonate	DFP
Dilute	dil.
Dioptre	D
Direct-coupled	DC
Direct current	d.c.
Dissociation constant, negative logarithm of	pK
Distilled	dist.
Dyne	dyn
Electrocardiogram	e.c.g.
Electroencephalogram	e.e.g.
Electromotive force	e.m.f.
Electron volt	eV
Electroretinogram	e.r.g.
End-plate potential	e.p.p.
Equation	eqn.
Equivalent (weight)	equiv
Experiment	expt.
but Experiment 4	Expt. 4 (Expts.)
Farad	F
Feet per second	ft./sec
Figure (illustration)	Fig. (Figs.)
Foot, feet	ft.
Foot-pound	ft.Lb.
Freezing-point	f.p.
γ-aminobutyric acid	GABA
Geiger–Müller	G–M
Giga- ($\times 10^9$)	G
Glomerular filtration rate	G.F.R.
Grain	gr.
Gram	g
Gram-calorie	cal
Gram-ion	g-ion
Gram-molecule	mole
Gravity, acceleration due to	*g*
Haemoglobin	Hb
Height	ht.
Henry	H
High frequency	h.f.
Horse-power	h.p.
Hour	hr
Hydrogen-ion concentration	$[H^+]$
Hydrogen-ion concentration, negative logarithm of	pH
5-hydroxytryptamine	5-HT
Inch	in.
Infra-red	I.R.

Insoluble	insol.
International unit	i.u.
Intra-arterial	I.A.
Intravenous	I.V.
Ion, e.g. sodium ion, sulphate ion	Na^+, SO_4^{2-}
Joule	J
Kilo- ($\times 10^3$), e.g. kilogram	k, e.g. kg
Kilocalorie	kcal
Kilowatt-hour	kWh
Laevo- (optical rotation)	(−)-
Laevo- (configuration)	L-
Liquid	liq.
Litre	l.
Logarithm to base e	ln
Logarithm to base 10	$\log_{10}$
Logarithm, when not used as a mathematical symbol	log.
Low frequency	l.f.
Lumen	lm
Maximal tubular reabsorption	Tm
Maximum	max.
Mega- ($\times 10^6$)	M
Megacycles per second	Mc/s
Megohm	MΩ
Melting-point	m.p.
Meta-	*m*-
Metre	m
Micro- ($\times 10^{-6}$), e.g. microgram	μ, e.g. μg (not γ)
μl. O_2/mg dry wt./hr	Q_{O_2}
Micron (10^{-6}m)	μ
Milli- ($\times 10^{-3}$), e.g. milligram	m, e.g. mg
Milli-equivalent	m-equiv
Millimicron	mμ
Millimolar (concentration)	mM
Millimole, -osmole	m-mole, -osmole
Milliunit	m-u.
Minimum	min.
Minute	min
Molal (concentration)	m
Molar (concentration)	M
Molar, with chemical formula	M-
Molecular weight	mol.wt.
Nano- ($\times 10^{-9}$)	n
National Physical Laboratory	N.P.L.
Normal (concentration)	N
Normal, with chemical formula	N-
Number	no.
but Number 4	No. 4 (Nos.)
Observed (in table headings)	Obs.
Ohm	Ω
Ortho-	*o*-
Page, pages	p., pp.
Para-	*p*-
Partial pressure, e.g. of O_2	pO_2
Per (e.g. metres per second)	/(e.g. m/sec)
Per cent	%
Pico- ($\times 10^{-12}$), e.g. picofarad	p, e.g. pF
Plate (illustration)	Pl. (Pls.)
Poise	P
Post-meridiem	p.m.
Potential difference	p.d.
Pound (mass)	lb.
Pound (force)	Lb.
Pounds per square inch	Lb./sq.in.
Precipitate (noun)	ppt.
Probability	*P*
Red blood corpuscle	R.B.C.
Relative band speed (chromatography)	R_F
Relative humidity	r.h.
Resistance-capacity (coupled)	RC
Respiratory quotient	R.Q.
Revolutions per minute	rev/min
Röntgen unit	r
Root mean square	r.m.s.
Second (unit of time)	sec
Secondary	*sec.*-
Soluble	sol.
Solution (table heads only)	Soln.
Specific gravity	sp.gr.
Specific heat	sp.ht.
Spinal segments	C6, L5, etc.
Square, e.g. square inch	sq. (e.g. sq.in.)
but square centimetre	cm^2
Standard deviation (about mean)	S.D. of an observation
Standard error (of a mean)	S.E. of mean
Standard temperature and pressure	s.t.p.
Standard wire gauge	S.W.G.
Temperature	temp.
Tera- ($\times 10^{12}$)	T
Tertiary	*tert.*-
Tetraethyl ammonium	TEA
Troland	td
Ultra-violet	U.V.
Unit	u.
Vapour density	v.d.
Vapour pressure	v.p.
Velocity	vel.
Volt	V
Volume	vol.
Watt	W
Weight	wt.
Yard	yd.
Year	yr

SYMBOLS NORMALLY USED TO DENOTE PARTICULAR QUANTITIES

These symbols are in accordance with the recommendations in the Report of the Symbols Committee of the Royal Society (1951). Note that all letters of the Latin alphabet are printed in italics.

Acceleration	a	Ionic strength	I
Activity	a	Length	l
Activity coefficient	f	Mass	m
Admittance	Y	Molar extinction coefficient	ϵ
Area	A	Molecular weight	M
Avogadro's number	N	No. of molecules	N
Boltzmann's constant	k	Number of moles	n
Capacitance	C	Osmotic pressure	Π
Charge (electric)	Q	Period	T
Charge of electron	$-e$	Potential; potential difference (electric)	V
Chemical potential	μ	Pressure	p
Conductance	G	Radius	r
Conductance, equivalent	Λ	Reactance	X
Conductivity	σ	Reflexion factor	ρ
Conductivity (electrochemistry)	κ	Refractive index	n
Current (electric)	I	Resistance (electrical)	R
Degree of dissociation	α	Resistivity	ρ
Density	ρ	Surface density of electric charge	σ
Diameter	d	Temperature (empirical)	θ
Diffusion coefficient	D	Temperature, absolute	T
Electrokinetic potential	ζ	Time	t
Electromotive force	E, V	Transmission factor (light)	τ
Equilibrium constant	K	Transport number	t
Faraday's constant	F	Valency	z
Force	F	Velocity	v, u
Frequency	f	Velocity, angular	ω
Gas constant per mole	R	Velocity constant	k
Gravitational acceleration	g	Viscosity	η
Height	h	Volume	V, v
Impedance	Z	Wave-length	λ
Inductance	L	Wave number	ν
Intensity (light)	I		

RECOMMENDED SPELLINGS FOR COMMONLY USED WORDS, ETC.

A priori (italic type)
After-effect
After-potential
Airtight
Amino acid
Analyse
Antidromic
Arteriovenous
Artifact
Axon
Band width
Base line
Blood flow, clot, pressure (but blood-pressure readings)
Body weight
Break-down (n), break down (v)
Breath-holding
Bromsulphalein
Caesarean (cap. C)
Callipers
Candela
Cation
Co-enzyme
Connexion
Co-ordinate
Costocervical
Cotton-wool
Counter-stain
Cover-slip
Cross-section (n)
Debris
Deoxy-
Deflexion
Disk
End-point, -plate
Enzymic
Egg white
Evans Blue
Filter paper
Flowmeter
Focused
Forearm, forefoot, forelimb

Fresh water (but fresh-water environment)
Guinea-pig
Half-life, -time
Hind foot, leg, limb
Homoeo-
Hydrolyse
Hypophysial
Indian ink (cap. I)
In situ, *in vitro*, etc. (italic type)
Iontophoresis
Isoelectric
Isoleucine
Judgement
Life cycle
Loudspeaker
Microchemical (but micro-organism)
Micro-electrode
Mid-brain
Midday, midway
Mid line
Miosis
Neuromuscular
Neurone
New-born
Non-homogeneous (NOT 'in-h.')
Non-myelinated (not un-)
Noradrenaline
One half, third
Over-active, -ity
Over-all (adj.), overalls (n.)
Pace-maker
Per se (italic type)
Perspex (cap. P)
Phlorrhizin
Photocell
Photo-electric
Post-ganglionic, -operative, etc.
Post mortem (Roman type, but 'at post-mortem')
Preganglionic, presynaptic
Pre-set, pre-treated, etc.
Radioactive, radiosodium
Radio-iodine
Rate-meter
Re-examine
Reflexion
Re-illuminate
Résumé
Role
Sea fish
Sea water (but sea-water environment)
Sense organ
Side arm
Soda-lime
Solutions: Krebs's, Locke's, Ringer's, Tyrode
Spinothalamic
Stopwatch
Student's (no quotes)
Subculture
Subfamily
Subnormal
Substance P
Supersensitive, -ity
Supraoptic
t test (no quotes)
Test-tube
Time constant, course
Tubocurarine (usually no D)
Ultrafilter
Un-ionized
Upstroke
Via
Vice versa (Roman type)
Watch-glass
Water-bath
Water-level
Watertight
Wave form, number
Wave-length, -front

PROOF CORRECTION

[Proof]

Results

Most of the experiments in the ~~the~~ present investigation were carried out with the use of cocaine and the results to be described ~~Hhere~~ were obtained with this drug unless special mention is made.

Magnitude of action potential

Conduction along the distal non-narcotised segment. When we take into consideration the finding of *Woronzow* that the size (the of) electric response suffers decrement along the distal region most beyond the depressed area, it is interesting, first of all, to ~~ascertain~~ whether the same would occur under present experimental conditions.

For this purpose dd leads were first employed. Records of propagated disturbances were taken ~~both~~ before and at varying intervals during narcosis and recovery. With ND leads diphasic records were obtained in which the POTENTIAL variations appearing in the narcotised and non narcotised distal regions are simultaneously represented in the two phases of each response, the first phase being derived from the former and the second from the latter.

Explanation of marks

1. Print in small capitals.
2. Delete letters or word indicated.
3. Invert letter.
4. Substitute a comma for the semi-colon.
5. The correct form of a jumbled word.
6. Insert a space.
7. Print in italics.
8. Indent as for a paragraph.
9. Print in roman type.
10, 11, 12. Methods of marking transposition; 10, of words within a line; 11, of a word from one line to another; 12, of letters within a word. If several words are out of order they should be numbered in correct order, and a transposition mark put in the margin.
13. Substitute one word for another.
14. Insert a word.

PROOF CORRECTION

[The same corrected]

RESULTS

Most of the experiments in the present investigation were carried out with the use of cocaine, and the results to be described below were obtained with this drug unless special mention is made.

Magnitude of action potential

Conduction along the distal non-narcotised segment. When we take into consideration the finding of Woronzow that the size of the electric response suffers most decrement along the distal region beyond the depressed area, it is interesting, first of all, to see whether the same would occur under the present experimental conditions. For this purpose DD leads were first employed. Records of propagated disturbances were taken both before and at varying intervals during narcosis and recovery.

With ND leads diphasic records were obtained in which the potential variations appearing in the narcotised and non-narcotised distal regions are simultaneously represented in the two phases of each response, the first phase being derived from the former and the second from the latter.

Explanation of marks (cont.)

15. Run on the type continuously, making no paragraph.
16. Print in capital letters.
17. Reduce the spaces in the line.
18. Substitute one letter for another.
19. Let the word crossed out stand.
20. Substitute good for battered type.
21. Begin a new paragraph.
22. Print in lower-case (small) letters.
23. Substitute a letter from the correct type fount.
24. Insert a hyphen.
25. Delete the hyphen, but leave a space between the words.
26. Align the type correctly.
27. Close up and delete the space.
28. Insert a full stop in place of the semi-colon.

A LIST OF PERIODICALS AND THEIR ABBREVIATIONS

This list includes all those periodicals to which reference was made at least twice during 1950–1960. The abbreviations generally follow those of the *World List of Scientific Periodicals* (Butterworth, 1952), whose rules should be consulted for titles not given here.

PERIODICAL	ABBREVIATION
Abhandlungen der Kgl. Sächsischen Gesellschaft (Akademie) der Wissenschaften. Math.-Phys. Kl. Leipzig	*Abh. sächs. Ges. (Akad.) Wiss.*
Abhandlungen zur exakten Biologie	*Abh. exakt. Biol.*
Abhandlungen zur theoretischen Biologie	*Abh. theoret. Biol.*
Abstracts of communications, XVIII international physiological congress	*Abstr. XVIII int. physiol. Congr.*
Acta allergologica, København	*Acta allerg., Kbh.*
Acta anatomica. Basel, New York	*Acta anat.*
Acta biologiae experimentalis	*Acta Biol. exp., Varsovie*
Acta biologica belgica	*Acta biol. belg.*
Acta botanica fennica	*Acta bot. fenn.*
Acta brevia neerlandica de physiologia, pharmacologia, microbiologia, etc.	*Acta brev. neerl. Physiol.*
Acta cardiologica. Bruxelles	*Acta cardiol., Brux.*
Acta chemica scandinavica	*Acta chem. scand.*
Acta clinica belgica	*Acta clin. belg.*
Acta endocrinologica. Bucarest	*Acta endocr., Bucarest*
Acta endocrinologica. Copenhagen	*Acta endocr., Copenhagen*
Acta endocrinologica (ucrainica). Kharkov	*Acta endocr., Kharkov*
Acta haematologica	*Acta haemat.*
Acta medica scandinavica	*Acta med. scand.*
Acta neurologica	*Acta neurol., Napoli*
Acta neurologica et psychiatrica belgica	*Acta neurol. belg.*
Acta obstetrica et gynecologica scandinavica	*Acta obstet. gynec. scand.*
Acta ophthalmologica	*Acta ophthal., Kbh.*
Acta oto-laryngologica	*Acta oto-laryng., Stockh.*
Acta oto-rhino-laryngologica belgica	*Acta oto-rhino-laryng. belg.*
Acta paediatrica belgica	*Acta paediat. belg.*
Acta paediatrica, Stockholm	*Acta paediat., Stockh.*
Acta pathologica et microbiologica scandinavica	*Acta path. microbiol. scand.*
Acta pharmacologica et toxicologica	*Acta pharm. tox., Kbh.*
Acta physiologica hungarica	*Acta physiol. hung.*
Acta physiologica latino-americana	*Acta physiol. latinoamer.*
Acta physiologica et pharmacologica neerlandica	*Acta physiol. pharm. neerl.*
Acta physiologica polonica	*Acta physiol. polon.*
Acta physiologica scandinavica	*Acta physiol. scand.*
Acta phytochimica. Tokyo	*Acta phytochim., Tokyo*
Acta psychiatrica et neurologica	*Acta psychiat., Kbh.*
Acta psychologica	*Acta psychol., Hague*
Acta radiologica	*Acta radiol., Stockh.*
Acta Scholae Medicinalis Universitatis Imperialis in Kioto	*Acta Sch. med. Univ. Kioto*
Acta Societatis medicorum Upsaliensis	*Acta Soc. Med. upsalien.*
Acta Societatis scientiarum Fennicae	*Acta Soc. Sci. fenn.*
Acta vitaminologiae	*Acta Vitamin., Wilno*
Acta vitaminologica. Milano	*Acta vitamin., Milano*
Acta zoologica	*Acta zool., Stockh.*
Actualités scientifiques et industrielles	*Actualités sci. industr.*
Advancement of Science (British Association reports)	*Advanc. Sci., Lond.*
Advances in Biological and Medical Physics	*Advanc. biol. med. Phys.*
Advances in Carbohydrate Chemistry	*Advanc. Carbohyd. Chem.*
Advances in Colloid Science	*Advanc. Colloid Sci.*
Advances in Enzymology and Related Subjects	*Advanc. Enzymol.*
Advances in Food Research	*Advanc. Food Res.*
Advances in Genetics	*Advanc. Genet.*
Advances in Internal Medicine	*Advanc. intern. Med.*
Advances in Protein Chemistry	*Advanc. Protein Chem.*

PERIODICAL	ABBREVIATION
Advances in Surgery	*Advanc. Surg.*
Agricultural Research Council Reports	*Agric. Res. Coun. Rep.*
American Chemical Journal	*Amer. chem. J.*
American Heart Journal	*Amer. Heart J.*
American Journal of Anatomy	*Amer. J. Anat.*
American Journal of Digestive Diseases (and Nutrition)	*Amer. J. dig. Dis.*
American Journal of Diseases of Children	*Amer. J. Dis. Child.*
American Journal of Hygiene	*Amer. J. Hyg.*
American Journal of Medicine	*Amer. J. Med.*
American Journal of Obstetrics and Gynecology	*Amer. J. Obstet. Gynec.*
American Journal of Ophthalmology	*Amer. J. Ophthal.*
American Journal of Pathology	*Amer. J. Path.*
American Journal of Pharmacy	*Amer. J. Pharm.*
American Journal of Physiology	*Amer. J. Physiol.*
American Journal of Psychiatry	*Amer. J. Psychiat.*
American Journal of Psychology	*Amer. J. Psychol.*
American Journal of the Medical Sciences	*Amer. J. med. Sci.*
American Journal of Roentgenology	*Amer. J. Roentgenol.*
American Journal of Tropical Diseases and Preventive Medicine	*Amer. J. trop. Dis.*
American Medical Association Archives of Pathology	*A.M.A. Arch. Path.*
American Review of Tuberculosis	*Amer. Rev. Tuberc.*
Anales del Instituto de biología	*An. Inst. Biol. Univ. Méx.*
Analyst	*Analyst*
Analytica chimica acta	*Analyt. chim. acta*
Analytical Chemistry	*Analyt. Chem.*
Anatomical Record	*Anat. Rec.*
Anatomischer Anzeiger	*Anat. Anz.*
Anesthesiology	*Anesthesiology*
Angeiologie	*Angeiologie*
Angewandte Chemie	*Angew. Chem.*
Angiology	*Angiology*
Animal Breeding Abstracts	*Anim. Breed. Abstr.*
Annaes da Faculdade de medicina de São Paulo	*Ann. Fac. Med. S. Paulo*
Annalen der Chemie	*Liebigs Ann.*
Annalen der Physik	*Ann. Phys., Lpz.*
Annales de biologie clinique	*Ann. Biol. clin.*
Annales de chimie	*Ann. Chim.*
Annales de chimie (et de physique)	*Ann. Chim. (Phys.)*
Annales de la nutrition et de l'alimentation	*Ann. Nutr., Paris*
Annales de la Société Royale des sciences médicales et naturelles de Bruxelles	*Ann. Soc. Sci. méd. nat. Brux.*
Annales de la Société Royale zoologique de Belgique	*Ann. Soc. zool. Belg.*
Annales de la Société zoologique et malacologique de Belgique	*Ann. Soc. zool. malac. Belg.*
Annales de l'Institut Pasteur	*Ann. Inst. Pasteur*
Annales de médecine	*Ann. Méd.*
Annales d'endocrinologie	*Ann. Endocrin., Paris*
Annales de physiologie et de physicochimie biologique	*Ann. Physiol. Physicochim. biol.*
Annales de physique	*Ann. Phys., Paris*
Annales d'oto-laryngologie	*Ann. Oto-laryng.*
Annales medicinae experimentalis et biologiae Fenniae	*Ann. Med. exp. Fenn.*
Annali d'igiene (sperimentale)	*Ann. Igiene (sper.)*
Annals of Allergy	*Ann. Allergy*
Annals of Applied Biology	*Ann. appl. Biol.*
Annals of Biochemistry and Experimental Medicine (Indian Institute for Medical Research)	*Ann. Biochem.*
Annals of Eugenics, London	*Ann. Eugen., Lond.*
Annals of Human Genetics	*Ann. human Genet.*
Annals of Internal Medicine	*Ann. intern. Med.*
Annals of Ophthalmology	*Ann. Ophthal.*
Annals of Otology, Rhinology and Laryngology	*Ann. Otol., etc., St Louis*
Annals of Physical Medicine	*Ann. phys. Med.*
Annals of Science	*Ann. Sci.*
Annals of Surgery	*Ann. Surg.*

PERIODICAL	ABBREVIATION
Annals of the New York Academy of Sciences	*Ann. N.Y. Acad. Sci.*
Annals of the Royal College of Surgeons of England	*Ann. R. Coll. Surg. Engl.*
Annals of Tropical Medicine and Parasitology	*Ann. trop. Med. Parasit.*
Année biologique	*Année biol.*
Année psychologique	*Année psychol.*
Annual Report. Industrial Fatigue Research Board, London	*A.R. industr. Fatig. Res. Bd., Lond.*
Annual Review of Biochemistry	*Annu. Rev. Biochem.*
Annual Review of Medicine	*Annu. Rev. Med.*
Annual Review of Microbiology	*Annu. Rev. Microbiol.*
Annual Review of Physiology	*Annu. Rev. Physiol.*
Annual Review of Plant Physiology	*Annu. Rev. Pl. Physiol.*
Annual Review of Psychology	*Annu. Rev. Psychol.*
Arbeitsphysiologie	*Arbeitsphysiologie*
Archiv der Pharmazie	*Arch. Pharm., Berl.*
Archiv für Anatomie und Physiologie	*Arch. Anat. Physiol., Lpz.*
Archiv für Anthropologie	*Arch. Anthrop., Braunschw.*
Archiv für Augenheilkunde	*Arch. Augenheilk.*
Archiv für die gesamte Physiologie	*Pflüg. Arch. ges. Physiol.*
Archiv für die gesamte Psychologie	*Arch. ges. Psychol.*
Archiv für Entwicklungsmechanik der Organismen	*Arch. EntwMech. Org.*
Archiv für experimentelle Pathologie und Pharmakologie	*Arch. exp. Path. Pharmak.*
Archiv für experimentellen Veterinärmedizin	*Arch. exp. Veterinärmed.*
Archiv für experimentelle Zellforschung	*Arch. exp. Zellforsch.*
Archiv für Gynaekologie	*Arch. Gynaek.*
Archiv für Hygiene	*Arch. Hyg., Berl.*
Archiv für Mikrobiologie	*Arch. Mikrobiol.*
Archiv für mikroskopische Anatomie (und Entwicklungsmechanik)	*Arch. mikr. Anat.*
Archiv für Ohren-, (Nasen- und Kehlkopf) heilkunde	*Arch. Ohr.-, Nas.- u. KehlkHeilk.*
Archiv für Ophthalmologie	*v. Graefes Arch. Ophthal.*
Archiv für pathologische Anatomie und Physiologie	*Virchows Arch.*
Archiv für physikalische Medizin	*Arch. phys. Med.*
Archiv für Verdauungskrankheiten	*Arch. VerdauKr.*
Archiv für wissenschaftiche u. praktische Tierheilkunde	*Arch. wiss. prakt. Tierheilk.*
Archives d'anatomie, d'histologie et d'embryologie	*Arch. Anat., Strasbourg*
Archives d'anatomie microscopique	*Arch. Anat. micr.*
Archives de biologie	*Arch. Biol., Paris*
Archives de l'Institut Pasteur d'Algérie	*Arch. Inst. Pasteur, Algér.*
Archives de médecine expérimentale et d'anatomie pathologique	*Arch. Méd. exp.*
Archives de physiologie normale et pathologique	*Arch. Physiol. norm. path.*
Archives de physique biologique	*Arch. Phys. biol.*
Archives des maladies du cœur, des vaisseaux et du sang	*Arch. Mal. Cœur*
Archives des sciences biologiques	*Arch. Sci. biol., St Pétersb.*
Archives des sciences physiologiques	*Arch. Sci. physiol.*
Archives d'ophtalmologie	*Arch. Ophtal., Paris*
Archives du Muséum d'histoire naturelle, Paris	*Arch. Hist. Mus. nat. Paris*
Archives générales de médicine	*Arch. gén. Méd.*
Archives internationales de biologie générale	*Biol. gén.*
Archives internationales de pharmacodynamie (et de thérapie)	*Arch. int. Pharmacodyn.*
Archives internationales de physiologie	*Arch. int. Physiol.*
Archives italiennes de biologie	*Arch. ital. Biol.*
Archives médico-chirurgicales de l'appareil respiratoroire	*Arch. méd.-chir. Appar. resp.*
Archives néerlandaises de physiologie de l'homme et des animaux	*Arch. néerl. Physiol.*
Archives of Biochemistry	*Arch. Biochem.* (after 1952). *Arch. Biochem. Biophys.*
Archives of Dermatology and Syphilology, New York	*Arch. Derm. Syph., N.Y.*
Archives of Disease in Childhood	*Arch. Dis. Childh.*
Archives of Industrial Health	*Arch. industr. Health*
Archives of Industrial Hygiene and Occupational Medicine	*Arch. industr. Hyg.*
Archives of Internal Medicine	*Arch. intern. Med.*
Archives of Neurology and Psychiatry. Chicago	*Arch. Neurol. Psychiat., Chicago*

PERIODICAL	ABBREVIATION
Archives of Neurology and Psychiatry. London	*Arch. Neurol. Psychiat., Lond.*
Archives of Ophthalmology	*Arch. Ophthal., N.Y.*
Archives of Otolaryngology, Chicago	*Arch. Otolaryng., Chicago*
Archives of Pathology (and Laboratory Medicine)	*Arch. Path. (Lab. Med.)*
Archives of Physical Therapy	*Arch. phys. Ther.*
Archives of Psychology	*Arch. Psychol., N.Y.*
Archives of Surgery. Chicago	*Arch. Surg., Chicago*
Archives of Surgery. London	*Arch. Surg., Lond.*
Archivio di biologia normale e patologica	*Sperimentale*
Archivio di farmacologia sperimentale e scienze affini	*Arch. Farmacol. sper.*
Archivio di fisiologia	*Arch. Fisiol.*
Archivio di patologia e clinica medica	*Arch. Pat. Clin. med.*
Archivio per le scienze mediche	*Arch. Sci. med.*
Archivos del Instituto de cardiologia de México	*Arch. Inst. Cardiol. Méx.*
Arkiv för kemi	*Ark. Kemi*
Atti della R. Accademia delle scienze	*Atti Accad. Torino*
Atti della R. Accademia Nazionale dei Lincei (Memorie)	*Mem. Accad. Lincei*
Atti della R. Accademia Nazionale dei Lincei (Rendiconti)	*R.C. Accad. Lincei*
Atti della Società lombarda di scienze mediche e biologiche	*Atti Soc. lombarda Sci. med. biol.*
Atti della Società medico-chirurgica di Padova	*Atti Soc. med.-chir. Padova*
Atti del R. Istituto veneto di scienze, lettere ed arti	*Atti Ist. veneto*
Australian and New Zealand Journal of Surgery	*Aust. N.Z. J. Surg.*
Australian Journal of Agricultural Research	*Aust. J. agric. Res.*
Australian Journal of Experimental Biology and Medical Science	*Aust. J. exp. Biol. med. Sci.* (after 1953) *Aust. J. exp. biol. Sci.*
Australian Journal of Psychology	*Aust. J. Psychol.*
Australian Journal of Scientific Research	*Aust. J. sci. Res.*
Australian Veterinary Journal	*Aust. vet. J.*
Bacteriological Review	*Bact. Rev.*
Behaviour	*Behaviour*
Beiträge zur Anatomie funktioneller Systeme	*Beitr. Anat. funkt. Syst.*
Beiträge zur chemischen Physiologie und Pathologie (Hofmeisters)	*Beitr. chem. Physiol. Path.*
Beiträge zur Geburtshilfe und Gynäkologie	*Beitr. Geburtsh. Gynäk.*
Beiträge zur pathologischen Anatomie und zur allgemeinen Pathologie	*Beitr. path. Anat.*
Bericht der Deutschen Chemischen Gesellschaft	*Ber. dtsch. chem. Ges.*
Berichte über die gesamte Physiologie und experimentelle Pharmakologie	*Ber. ges. Physiol.*
Bericht über die ophthalmologische Gesellschaft. Heidelberg	*Ber. ophth. Ges. Heidelberg*
Bericht über die Verhandlungen der Kgl. Sächsischen Gesellschaft (Akademie) der Wissenschaften zu Leipzig	*Ber sächs. Ges. (Akad.) Wiss.*
Berichte über die wissenschaftliche Biologie	*Ber. wiss. Biol.*
Berliner klinische Wochenschrift	*Berl. klin. Wschr.*
Bibliotek for læger	*Bibl. Læger*
Bibliotheca ophthalmologica	*Bibl. ophth.*
Biochemical Journal	*Biochem. J.*
Biochemical Pharmacoogy	*Biochem. Pharmacol.*
Biochemical Preparations	*Biochem. Prep.*
Biochemical Society Symposia	*Biochem. Soc. Symp.*
Biochemische Zeitschrift	*Biochem. Z.*
Biochimica	*Biochimica*
Biochimica et biophysica acta	*Biochim. biophys. acta*
Biofizika	*Biofizika*
Biologia generalis (Archives internationales de biologie générale)	*Biol. gen.*
Biologica Latina	*Biol. latina*
Biological Abstracts	*Biol. Abstr.*
Biological Bulletin of the Marine Biological Laboratory, Woods Hole	*Biol. Bull., Woods Hole*
Biological Reviews	*Biol. Rev.*
Biological Symposia	*Biol. Symp.*
Biologisches Zentralblatt	*Biol. Zbl.*

PERIODICAL	ABBREVIATION
Biologiske Meddelelser. K. Danske Videnskabernes Selskab	*Biol. Medd., Kbh.*
Biometrika. Cambridge	*Biometrika*
Biophysics (in English)	*Biophys., Lond.*
Blood. The Journal of Hematology	*Blood*
Bollettino della Società italiana di biologia sperimentale	*Boll. Soc. ital. Biol. sper.*
Brain	*Brain*
British Abstracts AIII—Physiology, Biochemistry, Anatomy	*Brit. Abstr.* AIII
British Association Reports	*Advanc. Sci., Lond.*
British Chemical and Physiological Abstracts	*Brit. chem. physiol. Abstr.*
British Dental Journal	*Brit. dent. J.*
British Heart Journal	*Brit. Heart J.*
British Journal of Animal Behaviour	*Brit. J. Anim. Behav.*
British Journal of Cancer	*Brit. J. Cancer*
British Journal of Children's Diseases	*Brit. J. Child. Dis.*
British Journal of Experimental Biology	*Brit. J. exp. Biol.*
British Journal of Experimental Pathology	*Brit. J. exp. Path.*
British Journal of Haematology	*Brit. J. Haemat.*
British Journal of Industrial Medicine	*Brit. J. industr. Med.*
British Journal of Nutrition	*Brit. J. Nutr.*
British Journal of Ophthalmology	*Brit. J. Ophthal.*
British Journal of Pharmacology and Chemotherapy	*Brit. J. Pharmacol.*
British Journal of Physical Medicine and Industrial Hygiene	*Brit. J. phys. Med.*
British Journal of Physiological Optics	*Brit. J. physiol. Optics*
British Journal of Plastic Surgery	*Brit. J. plast. Surg.*
British Journal of Psychology	*Brit. J. Psychol.*
British Journal of Radiology. New Series	*Brit. J. Radiol. N.S.*
British Journal of Surgery	*Brit. J. Surg.*
British Medical Bulletin	*Brit. med. Bull.*
British Medical Journal	*Brit. med. J.*
British Science News (British Council)	*Brit. Sci. News*
British Veterinary Journal	*Brit. vet. J.*
Bulletin biologique de la France et de la Belgique	*Bull. biol.*
Bulletin. Council for Scientific and Industrial Research	*Bull. Coun. sci. industr. Res. Aust.*
Bulletin. Cranbrook Institute of Science	*Bull. Cranbrook Inst. Sci.*
Bulletin de biologie et de médecine experimentale de l'U.R.S.S.	*Bull. Biol. Méd. exp. U.R.S.S.*
Bulletin de l'Académie de médecine de Belgique	*Bull. Acad. Méd. Belg.*
Bulletin de l'Académie de médecine	*Bull. Acad. Méd., Paris*
Bulletin de l'Académie nationale de médecine	*Bull. Acad. nat. Méd.*
Bulletin de la Société chimique de Paris	*Bull. Soc. chim. Paris*
Bulletin de la Société de chimie biologique	*Bull. Soc. Chim. biol., Paris*
Bulletin de la Société scientifique et Station biologique d'Arcachon	*Bull. Soc. sci. Arcachon*
Bulletin d'histologie appliquée à la physiologie, etc.	*Bull. Histol. Tech. micr.*
Bulletin et Mémoires de la Société médicale des hôpitaux de Paris	*Bull. Soc. méd. Hôp. Paris*
Bulletin. Missouri Agricultural Experimental Station	*Bull. Mo. agric. Exp. Sta.*
Bulletin of Entomological Research	*Bull. ent. Res.*
Bulletin of Mathematical Biophysics	*Bull. math. Biophys.*
Bulletin of the Museum of Comparative Zoology	*Bull. Mus. comp. Zool.*
Bulletin of the Neurological Institute of New York	*Bull. neurol. Inst. N.Y.*
Bulletin of the United States Bureau of Standards	*Bull. U.S. Bur. Stand.*
Bulletin of the World Health Organisation	*Bull. World Hlth Org.*
Bulletin of War Medicine	*Bull. War Med.*
Bulletin. Société chimique de France	*Bull. Soc. chim. Fr.*
Bulletin. U.S. Army Medical Department	*Bull. U.S. Army med. Dep.*
Bureau of Standards Journal of Research	*Bur. Stand. J. Res., Wash.*
California and Western Medicine	*Calif. West. Med.*
Canadian Journal of Biochemistry and Physiology	*Canad. J. Biochem. Physiol.*
Canadian Journal of Medical Sciences	*Canad. J. med. Sci.*
Canadian Journal of Medicine and Surgery	*Canad. J. Med. Surg.*
Canadian Journal of Research: Zoology	*Canad. J. Res., Zool.*

Periodical	Abbreviation
Canadian Medical Association Journal	*Canad. med. Ass. J.*
Cancer Research	*Cancer Res.*
Cardiologia, Basle	*Cardiologia, Basle*
Cellule	*Cellule*
Chemical Abstracts	*Chem. Abstr.*
Chemické Listy pro Vědu a Průmysl	*Chem. Listy*
Chemische Zeitschrift	*Chem. Z.*
Chemistry and Industry (Review)	*Chem. & Ind. (Rev.)*
Chinese Journal of Physiology	*Chin. J. Physiol.*
Chinese Journal of Physiology. Report Series	*Chin. J. Physiol. Rep. Ser.*
Chromosoma	*Chromosoma*
Circular. Missouri Agricultural Experiment Station	*Circ. Mo. agric. Exp. Sta.*
Circulation	*Circulation*
Circulation Research	*Circulation Res.*
Clinical Science	*Clin. Sci.*
Clinics. Philadelphia	*Clinics*
Cold Spring Harbor Symposia on Quantitative Biology	*Cold Spr. Harb. Symp. quant. Biol.*
Colston Papers	*Colston Papers*
Comptes rendus de l'Académie des sciences de l'U.R.S.S.	*C.R. Acad. Sci. U.R.S.S.*
Comptes rendus des travaux du Laboratoire de Carlsberg	*C.R. Lab. Carlsberg*
Comptes rendus hebdomadaires des séances de l'Académie des Sciences	*C.R. Acad. Sci., Paris*
Comptes rendus hebdomadaires des séances et mémoires de la Société de biologie	*C.R. Soc. Biol., Paris*
Contributions to Embryology. (Publications of the Carnegie Institution)	*Contr. Embryol. Carneg. Instn*
Cornell Veterinarian	*Cornell Vet.*
Current Science	*Curr. Sci.*
Deep-Sea Research	*Deep-Sea Res.*
Dental Record	*Dent. Rec.*
Dermatologica, Basel	*Dermatologica, Basel*
Deutsche medizinische Wochenschrift	*Dtsch. med. Wschr.*
Deutsche Monatsschrift für Zahnheilkunde	*Dtsch. Mschr. Zahnheilk.*
Deutsche Zeitschrift für Nervenheilkunde	*Dtsch. Z. Nervenheilk.*
Diabetes	*Diabetes*
Discussions of the Faraday Society	*Disc. Faraday Soc.*
Documenta ophthalmologica	*Docum. ophthal.*
Doklady Akademii Nauk	*C.R. Acad. Sci. U.R.S.S.*
Edinburgh Medical Journal	*Edinb. med. J.*
Electroencephalography and Clinical Neurophysiology	*Electroenceph. clin. Neurophysiol.*
Electronic Engineering	*Electron. Engng*
Endeavour	*Endeavour*
Endocrinologie Japonaise	*Endocrin. Japon.*
Endocrinology	*Endocrinology*
Endokrinologie	*Endokrinologie*
Enzymologia	*Enzymologia*
Ergebnisse der Anatomie und Entwicklungsgeschichte	*Ergebn. Anat. EntwGesch.*
Ergebnisse der Biologie	*Ergebn. Biol.*
Ergebnisse der Enzymforschung	*Ergebn. Enzymforsch.*
Ergebnisse der Physiologie	*Ergebn. Physiol.*
Ergebnisse der Vitamin- u. Hormonforschung	*Ergebn. Vitam.- u. Hormonforsch.*
Études néo-natales	*Étud. néonatales*
Eugenics Review	*Eugen. Rev.*
Excerpta Medica. Amsterdam	*Excerpta med., Amst.*
Excerpta Medica. Basel, Leipzig	*Excerpta med., Basel*
Experientia	*Experientia*
Experimental Cell Research	*Exp. Cell Res.*
Experimental Medicine and Surgery	*Exp. Med. Surg.*
Experimental Neurology	*Exp. Neurol.*
Farmacista italiano	*Farm. ital., Roma*
Farmacista italiano e medico pratico	*Farm. ital., Napoli*
Farmaco	*Farmaco*

PERIODICAL	ABBREVIATION
Federation Proceedings	*Fed. Proc.*
Fermentforschung	*Fermentforschung*
Fiziologicheskii Zhurnal (Moscow)	*Sechenov J. Physiol.*
Folia haematologica	*Folia haemat., Lpz.*
Folia pharmacologica japonica	*Folia pharm. jap.*
Folia psychiatrica, neurologica et neurochirurgica neerlandica	*Folia psychiat. neerl.*
Food Research	*Food Res.*
Gastroenterologia, Basel	*Gastroenterologia, Basel*
Gastroenterology	*Gastroenterology*
Genetics	*Genetics*
Geographical Review	*Geogr. Rev.*
Glasgow Medical Journal	*Glasgow med. J.*
Growth	*Growth*
Guy's Hospital Reports	*Guy's Hosp. Rep.*
Gynécologie et obstétrique	*Gynéc. et Obstét.*
Haematologica	*Haematologica*
Handbuch der Biochemie	*Handb. Biochem., Berlin*
Handbuch der biochemischen Arbeitsmethoden	*Handb. biochem. ArbMeth.*
Handbuch der biologischen Arbeitsmethoden	*Handb. biol. ArbMeth.*
Handbuch der vergleichenden Physiologie	*Handb. vergl. Physiol.*
Harvey Lectures	*Harvey Lect.*
Heart	*Heart*
Helvetica chimica acta	*Helv. chim. acta*
Helvetica paediatrica acta	*Helv. paediat. acta*
Helvetica physiologica et pharmacologica acta	*Helv. physiol. acta*
Hereditas, Lund	*Hereditas, Lund*
Heredity. An international journal of genetics	*Heredity*
Hoppe-Seyler's Zeitschrift für physiologische Chemie	*Hoppe-Seyl. Z.*
Hormone. Oss, Holland	*Hormone*
Hormoner. Stockholm	*Hormoner*
Hormones. London	*Hormones*
Hungarica acta physiologica	*Hung. acta physiol.*
Index medicus. Washington	*Index medicus*
Indian Journal of Medical Research	*Indian J. med. Res.*
Indian Journal of Veterinary Science and Animal Husbandry	*Indian J. vet. Sci.*
Industrial Engineering Chemistry (Analytical)	*Indust. Engng Chem. (Anal.)*
International Abstract of Biological Sciences	*Int. Abstr. biol. Sci.*
International Archives of Allergy, Basel	*Int. Arch. Allergy, Basel*
International Archives of Allergy and Applied Immunology. New York	*Int. Arch. Allergy, N.Y.*
International Journal of Radiation Biology	*Int. J. rad. Biol.*
International Review of Cytology	*Int. Rev. Cytol.*
International Review of Neurobiology	*Int. Rev. Neurobiol.*
Interstate Medical Journal, St Louis	*Interst. med. J.*
Irish Journal of Medical Science	*Irish J. med. Sci.*
Jahresbericht Physiologie und experimentelle Pharmakologie	*Jber. Physiol. exp. Pharm.*
Japanese Journal of Medical Sciences	*Jap. J. med. Sci.*
Part 2. Biochemistry	*Biochem.*
Part 3. Biophysics	*Biophys.*
Japanese Journal of Physiology	*Jap. J. Physiol.*
Johns Hopkins Hospital Bulletin	*Johns Hopk. Hosp. Bull.*
Johns Hopkins Hospital Reports	*Johns Hopk. Hosp. Rep.*
Journal de chimie physique (et Revue générale des colloides), électrochimie, thermochimie, radiochimie, mécanique chimique, stœchiométrie	*J. Chim. phys.*
Journal de l'anatomie et de la physiologie	*J. Anat., Paris*
Journal de pharmacie et de chimie	*J. Pharm. Chim., Paris*
Journal de physiologie (et de pathologie générale)	*J. Physiol. Path. gén.* (after 1953) *J. Physiol., Paris*

PERIODICAL	ABBREVIATION
Journal de psychologie normale et pathologique	*J. Psychol. norm. path.*
Journal für Psychologie und Neurologie	*J. Psychol. Neurol., Lpz.*
Journal-Lancet, Minneapolis	*Jl-Lancet*
Journal of Agricultural Research. Washington	*J. agric. Res.*
Journal of Agricultural Science. Cambridge	*J. agric. Sci.*
Journal of Allergy	*J. Allergy*
Journal of Anatomy (and Physiology)	*J. Anat., Lond.*
Journal of Animal Ecology	*J. Anim. Ecol.*
Journal of Animal Science	*J. Anim. Sci.*
Journal of Applied Physiology	*J. appl. Physiol.*
Journal of Aviation Medicine	*J. Aviat. Med.*
Journal of Bacteriology	*J. Bact.*
Journal of Biochemistry	*J. Biochem. Tokyo*
Journal of Biological Chemistry	*J. biol. Chem.*
Journal of Biophysical and Biochemical Cytology	*J. biophys. biochem. Cytol.*
Journal of Biophysics	*J. Biophys., Tokyo*
Journal of Bone and Joint Surgery	*J. Bone Jt. Surg.*
Journal of Cancer. London	*J. Cancer, Lond.*
Journal of Cancer Research. Baltimore	*J. Cancer Res.*
Journal of Cellular and Comparative Physiology	*J. cell. comp. Physiol.*
Journal of Chemical Physics	*J. chem. Phys.*
Journal of Clinical Endocrinology	*J. clin. Endocrin.*
Journal of Clinical Investigation	*J. clin. Invest.*
Journal of Clinical Pathology	*J. clin. Path.*
Journal of Clinical Research	*J. clin. Res.*
Journal of Colloid Science	*J. Colloid Sci.*
Journal of Comparative and Physiological Psychology	*J. comp. physiol. Psychol.*
Journal of Comparative Neurology (and Psychology)	*J. comp. Neurol.*
Journal of Cytochemistry	*J. Cytochem.*
Journal of Dairy Research	*J. Dairy Res.*
Journal of Dairy Science	*J. Dairy Sci.*
Journal of Dental Research	*J. dent. Res.*
Journal of Economic Entomology	*J. econ. Ent.*
Journal of Embryology and Experimental Morphology	*J. Embryol.*
Journal of Endocrinology	*J. Endocrin.*
Journal of Experimental Biology	*J. exp. Biol.*
Journal of Experimental Medicine	*J. exp. Med.*
Journal of Experimental Psychology	*J. exp. Psychol.*
Journal of Experimental Zoology	*J. exp. Zool.*
Journal of General Microbiology	*J. gen. Microbiol.*
Journal of General Physiology	*J. gen. Physiol.*
Journal of General Psychology	*J. gen. Psychol.*
Journal of Genetics	*J. Genet.*
Journal of Gerontology	*J. Geront.*
Journal of Histochemistry	*J. Histochem.*
Journal of Histochemistry and Cytochemistry	*J. Histochem. Cytochem.*
Journal of Hygiene	*J. Hyg., Camb.*
Journal of Immunology	*J. Immunol.*
Journal of Industrial Hygiene (and Toxicology)	*J. industr. Hyg.*
Journal of Infectious Diseases	*J. infect. Dis.*
Journal of Insect Physiology	*J. insect Physiol.*
Journal of Investigative Dermatology	*J. invest. Derm.*
Journal of Laboratory and Clinical Medicine	*J. Lab. clin. Med.*
Journal of Laryngology (Rhinology) and Otology	*J. Laryng.*
Journal of Medical Research	*J. med. Res.*
Journal of Metabolic Research	*J. metab. Res.*
Journal of Molecular Biology	*J. mol. Biol.*
Journal of Morphology	*J. Morph.*
Journal of Neurochemistry	*J. Neurochem.*
Journal of Neurology and Psychopathology	*J. Neurol. Psychopath.*
Journal of Neurology (Neurosurgery) and Psychiatry	*J. Neurol.*
Journal of Neuropathology and Experimental Neurology	*J. Neuropath.*
Journal of Neurophysiology	*J. Neurophysiol.*
Journal of Neurosurgery	*J. Neurosurg.*

PERIODICAL	ABBREVIATION
Journal of Nutrition	*J. Nutr.*
Journal of Obstetrics and Gynaecology of the British Empire	*J. Obstet. Gynaec., Brit. Emp.*
Journal of Organic Chemistry	*J. org. Chem.*
Journal of Pathology and Bacteriology	*J. Path. Bact.*
Journal of Pharmacology and Experimental Therapeutics	*J. Pharmacol.*
Journal of Pharmacy and Pharmacology	*J. Pharm., Lond.*
Journal of Physical and Colloid Chemistry	*J. phys. Chem.*
Journal of Physiology	*J. Physiol.*
Journal of Physiology, U.S.S.R. (in English)	*Sechenov J. Physiol., Lond.*
Journal of Psychology	*J. Psychol.*
Journal of Research. National Bureau of Standards	*J. Res. nat. Bur. Stand.*
Journal of Scientific Instruments	*J. sci. Instrum.*
Journal of the Acoustical Society of America	*J. acoust. Soc. Amer.*
Journal of the American Chemical Society	*J. Amer. chem. Soc.*
Journal of the American Medical Association	*J. Amer. med. Ass.*
Journal of the American Pharmaceutical Association	*J. Amer. pharm. Ass.*
Journal of the American Veterinary Medical Association	*J. Amer. vet. med. Ass.*
Journal of the Canadian Medical Association	*J. Canad. med. Ass.*
Journal of the Chemical Society	*J. chem. Soc.*
Journal of the Faculty of Radiologists, London	*J. Fac. Radiol., Lond.*
Journal of the Institution of Electrical Engineers	*J. Instn elect. Engrs*
Journal of the Japanese Biochemical Society	*J. Jap. biochem. Soc.*
Journal of the Marine Biological Association	*J. Mar. biol. Ass. U.K.*
Journal of the Mount Sinai Hospital	*J. Mt Sinai Hosp.*
Journal of the National Cancer Institute	*J. nat. Cancer Inst.*
Journal of the Optical Society of America	*J. opt. Soc. Amer.*
Journal of the Pharmaceutical Society of Japan	*J. pharm. Soc. Japan*
Journal of the Physiological Society of Japan	*J. physiol. Soc. Japan*
Journal of the Royal Army Medical Corps	*J. R. Army med. Corps*
Journal of the Royal Microscopical Society	*J. R. micr. Soc.*
Journal of the Royal Statistical Society	*J. R. statist. Soc.*
Journal of the South African Veterinary Medical Association	*J. S. Afr. Vet. Med. Ass.*
Journal of the Textile Institute	*J. Text. Inst.*
Journal of Urology	*J. Urol.*
Klinische Wochenschrift	*Klin. Wschr.*
Kolloidzeitschrift	*Kolloidzschr.*
Kongelige Danske Videnskabernes Selskabs Skrifter	*K. danske vidensk. Selsk.*
Lancet	*Lancet*
Laryngoscope	*Laryngoscope*
Liebigs Annalen der Chemie	*Liebigs Ann.*
Luftfahrtmedizin	*Luftfahrtmedizin*
Medical Journal of Australia	*Med. J. Aust.*
Medical Journal of South Africa	*Med. J. S. Afr.*
Medical Science Abstracts and Reviews	*Med. Sci. Abstr.*
Medicine	*Medicine*
Medico-Chirurgical Transactions	*Med.-chir. Trans.*
Mémoires couronnés et autres mémoires p.p. l'Académie R. des sciences (etc.)	*Mém. cour. Acad. R. Belg.*
Memoirs of the Society for Endocrinology	*Mem. Soc. Endocrin.*
Memoirs of the Wistar Institute of Anatomy	*Mem. Wistar Inst. Anat.*
Metabolism	*Metabolism*
Methods in Medical Research	*Methods med. Res.*
Mikrochemie	*Mikrochemie*
Mikroscopie	*Mikroscopie*
Minnesota Medicine	*Minn. Med.*
Mitteilungen aus den Grenzgebieten der Medizin und Chirurgie	*Mitt. Grenzgeb. Med. Chir.*
Mitteilungen aus Finsens Medicinske Lysinstitut in Kopenhagen	*Mitt. Finsens med. Lysinst.*
Monatschrift für Psychiatrie und Neurologie	*Mschr. Psychiat. Neurol.*
Monatshefte für Chemie und verwandte Teile anderer Wissenschaften	*Mh. Chem.*

PERIODICAL	ABBREVIATION
Monatsschrift für Kinderheilkunde	*Mschr. Kinderheilk.*
Monitore zoologico italiano	*Monit. zool. ital.*
Morgagni	*Morgagni*
Morphologisches Jahrbuch	*Morph. Jb.*
Münchener medizinische Wochenschrift	*Münch. med. Wschr.*
Nature	*Nature, Lond.*
Naturwissenschaften	*Naturwissenschaften*
Nederlandsch tijdschrift voor geneeskunde	*Ned. Tijdschr. Geneesk.*
Neurology	*Neurology*
Névraxe	*Névraxe*
New England Journal of Medicine	*New Engl. J. Med.*
New Phytologist	*New Phytol.*
New Zealand Medical Journal	*N.Z. med. J.*
Nordisk Medicin	*Nord. Med.*
Nordisk medicinsk Tidskrift	*Nord. med. Tidskr.*
Nordiskt medicinskt Arkiv	*Nord. med. Arkiv*
Nouvelles archives italiennes de biologie	*Nouv. Arch. ital. Biol.*
Nucleonics	*Nucleonics*
Nutrition	*Nutrition, Lond.*
Nutrition. Annales cliniques, biologiques, thérapeutiques	*Nutrition, Paris*
Nutrition Abstracts and Reviews	*Nutr. Abstr. Rev.*
Nutrition Reviews	*Nutr. Rev.*
Obstétrique et gynécologie	*Obstét. et Gynéc.*
Ohio Journal of Science	*Ohio J. Sci.*
Onderstepoort Journal of Veterinary Science and Animal Industry	*Onderstepoort J. vet. Sci.*
Ophthalmologica, Basel	*Ophthalmologica, Basel*
Oppenheimer's Handbuch der Biochemie	*Handb. Biochem., Berl.*
Optica acta	*Optica acta*
Parasitology	*Parasitology*
Pediatrics	*Pediatrics*
Pflügers Archiv	*Pflüg. Arch. ges. Physiol.*
Pharmaceutica Acta Helvetiae	*Pharm. acta Helvet.*
Pharmaceutical Journal	*Pharm. J.*
Pharmacological Reviews	*Pharmacol. Rev.*
Philosophical Magazine	*Phil. Mag.*
Philosophical Transactions of the Royal Society	*Phil. Trans.*
Physica	*Physica*
Physics in Medicine and Biology	*Physics in Med. Biol.*
Physikalische Zeitschrift	*Phys. Z.*
Physiologia Bohemoslovenica	*Physiol. Bohem.*
Physiologia comparata et oecologia	*Physiol. comp.*
Physiological Abstracts	*Physiol. Abstr.*
Physiological Reviews	*Physiol. Rev.*
Physiological Zoölogy	*Physiol. Zoöl.*
Physiologie	*Physiologie*
Physiologist	*Physiologist*
Phytopathologische Zeitschrift	*Phytopath. Z.*
Phytopathology	*Phytopathology*
Plant Physiology	*Plant Physiol.*
Policlinico	*Policlinico*
Post Graduate Medical Journal, London	*PostGrad. Med. J.*
Presse médicale	*Pr. médt.*
Problemy Fiziologicheskoi Optiki	*Probl. physiol. Opt., Leningr.*
Proceedings of the American Philosophical Society	*Proc. Amer. phil. Soc.*
Proceedings of the Chicago Institute of Medicine	*Proc. Chicago Inst. Med.*
Proceedings of the Imperial Academy	*Proc. imp. Acad. Japan*
Proceedings of the Institute of Radio Engineers. New York	*Proc. Inst. Radio Engrs, N.Y.*
Proceedings of the Mayo Clinic	*Proc. Mayo Clin.*
Proceedings of the National Academy of Sciences, Washington	*Proc. nat. Acad. Sci., Wash.*
Proceedings of the Nutrition Society	*Proc. Nutr. Soc.*
Proceedings of the Physical Society	*Proc. phys. Soc. Lond.*
Proceedings of the Physiological Society	*Proc. physiol. Soc.*

PERIODICAL	ABBREVIATION
Proceedings of the Royal Academy of Sciences, Amsterdam	*Proc. Acad. Sci. Amst.*
Proceedings of the Royal Dublin Society	*Proc. R. Dublin Soc.*
Proceedings of the Royal Society	*Proc. Roy. Soc.*
Proceedings of the Royal Society of Canada	*Proc. Roy. Soc. Can.*
Proceedings of the Royal Society of Edinburgh	*Proc. Roy. Soc. Edinb.*
Proceedings of the Royal Society of Medicine	*Proc. R. Soc. Med.*
Proceedings of the Royal Society of Queensland, Brisbane	*Proc. Roy. Soc. Qd*
Proceedings of the Royal Society of Victoria, Melbourne	*Proc. Roy. Soc. Vict.*
Proceedings of the Society of Experimental Biology and Medicine	*Proc. Soc. exp. Biol., N.Y.*
Proceedings of the University of Otago Medical School	*Proc. Univ. Otago med. Sch.*
Proceedings of the Zoological Society of London	*Proc. zool. Soc. Lond.*
Progress in Biophysics and Biophysical Chemistry	*Progr. Biophys.*
Progress in Neurology and Psychiatry	*Progr. Neurol. Psychiat.*
Protoplasma	*Protoplasma*
Psychological Review	*Psychol. Rev.*
Psychosomatic Medicine	*Psychosom. Med.*
Pubblicazioni della Stazione zoologica di Napoli	*Pubbl. Staz. zool. Napoli*
Publications. Carnegie Institution of Washington	*Publ. Carneg. Instn*
Publications of the South African Institute for Medical Research	*Publ. S. Afr. Inst. med. Res.*
Quarterly Bulletin of the Health Organization, League of Nations	*Quart. Bull. Hlth Org. L.o.N.*
Quarterly Journal of Experimental Physiology	*Quart. J. exp. Physiol.*
Quarterly Journal of Experimental Psychology	*Quart. J. exp. Psychol.*
Quarterly Journal of Medicine	*Quart. J. Med.*
Quarterly Journal of Microscopical Science	*Quart. J. micr. Sci.*
Quarterly Journal of Pharmacy and Allied Sciences	*Quart. J. Pharm.*
Quarterly Review of Biology	*Quart. Rev. Biol.*
Radiology	*Radiology*
Recent Progress in Hormone Research	*Recent Progr. Hormone Res.*
Recueil des travaux chimiques des Pays-Bas et de la Belgique	*Rec. Trav. chim. Pays-Bas*
Rendiconti della R. Accademia Nazionale dei Lincei	*R.C. Accad. Lincei*
Rendiconti delle sessioni della R. Accademia delle scienze dell' Instituto di Bologna	*R. C. Accad. Bologna*
Rendiconti Scientifici Farmitalia	*R. C. sci. farmital.*
Report. Industrial Health Research Board. London	*Rep. industr. Hlth Res. Bd, Lond.*
Report of the Institute of Animal Pathology, University of Cambridge	*Rep. Inst. Anim. Path. Univ. Camb.*
Report of the British Association for the Advancement of Science	*Rep. Brit. Ass.* (after 1938) *Advanc. Sci. Lond.*
Report on Progress in Physics	*Rep. Progr. Phys.*
Report on the Progress of Chemistry	*Rep. Progr. Chem.*
Report to the Local Government Board on Public Health and Medical Matters	*Rep. loc. Govt. Bd. publ. Hlth*
Research	*Research, Lond.*
Research Bulletin. Missouri Agricultural Experiment Station	*Res. Bull. Mo. agric. Exp. Sta.*
Research Publications. Association for Research in Nervous and Mental Diseases	*Res. Publ. Ass. nerv. ment. Dis.*
Review of Scientific Instruments	*Rev. sci. Instrum.*
Revista de la Asociación médica argentina	*Rev. Asoc. méd. argent.*
Revista de la sociedad argentina de biología	*Rev. Soc. argent. Biol.*
Revista española de fisiología	*Rev. esp. Fisiol.*
Revista médica latinoamericana	*Rev. méd. lat.-amer.*
Revue belge de pathologie et de médecine expérimentale	*Rev. belge Path.*
Revue canadienne de biologie	*Rev. canad. Biol.*
Revue des archives italiennes de biologie	*Rev. Arch. ital. Biol.*
Revue d'hématologie	*Rev. Hémat.*
Revue d'optique	*Rev. Opt.* (*théor. instrum.*)
Revue française d'endocrinologie	*Rev. franç. Endocr.*
Revue générale d'Histologie	*Rev. gén. Histol.*
Revue médicale de la Suisse romande	*Rev. méd. Suisse rom.*

PERIODICAL	ABBREVIATION
Revue médicale de Nancy	*Rev. méd. Nancy*
Revue neurologique	*Rev. Neurol.*
Riforma medica	*Rif. med.*
Rivista di biologia	*Riv. Biol.*
Russkii Fiziologicheskii Zhurnal	*Russk. fiz. Zh.* (after 1932) *Sechenov J. Physiol.*
Scandinavian Journal of Clinical and Laboratory Investigation	*Scand. J. clin. Lab. Invest.*
Schweizer Archiv für Neurologie und Psychiatrie	*Schweiz Arch. Neurol. Psychiat.*
Schweizerische medizinische Wochenschrift	*Schweiz. med. Wschr.*
Science	*Science*
Science Abstracts	*Sci. Abstr.*
Science Progress	*Sci. Progr.*
Science Progress in the Twentieth Century	*Sci. Progr. Twent. Cent.*
Scientific Monthly, New York	*Sci. Mon. N.Y.*
Scientific Papers of the U.S. Bureau of Standards	*Sci. Pap. U.S. Bur. Stand.*
Scottish Medical Journal	*Scot. med. J.*
Shirley Institute Memoirs	*Shirley Inst. Mem.*
Sitzungsberichte der Kgl. Preussischen Akademie der Wissenschaft zu Berlin	*S.B. preuss. Akad. Wiss.*
Skandinavisches Archiv für Physiologie	*Skand. Arch. Physiol.*
South African Journal of Medical Sciences	*S. Afr. J. med. Sci.*
South African Journal of Science	*S. Afr. J. Sci.*
South African Medical Journal	*S. Afr. med. J.*
Special Report Series, Medical Research Council, London	*Spec. Rep. Ser. med. Res. Coun., Lond.*
Sperimentale. Archivio di biologia normale e patologica	*Sperimentale*
Stain Technology	*Stain Tech.*
Stanford Medical Bulletin	*Stanf. med. Bull.*
Stanford University Publications—University Series Biological Sciences Medical Sciences	*Stanford Univ. Publ.* *biol. Sci.* *med. Sci.*
Strahlentherapie	*Strahlentherapie*
Suomalaisen Tiedeakatemian Toimituksia, Helsinki	*Ann. Acad. Sci. fenn.*
Surgery	*Surgery*
Surgery, Gynecology and Obstetrics	*Surg. Gynec. Obstet.*
Surgical Forum	*Surg. Forum*
Svensk kemisk Tidskrift	*Svensk kem. Tidskr.*
Symposia of the Society for Experimental Biology	*Symp. Soc. exp. Biol.*
Tabulae biologicae	*Tabul. biol., Amst.*
Tabulae biologicae periodicae	*Tabul. biol., Hague*
Technical Report of the Advisory Committee for Aeronautics	*Tech. Rep. adv. Comm. Aero., Lond.*
Technical Report Series. World Health Organisation	*Tech. Rep. Wld Hlth Org.*
Texas Reports on Biology and Medicine	*Texas Rep. Biol. Med.*
Thorax	*Thorax*
Tohoku Journal of Experimental Medicine	*Tohoku J. exp. Med.*
Toxicology and Applied Pharmacology	*Toxicol. appl. Pharmacol.*
Trabajos del Instituto Cajal de investigaciones biológicas	*Trab. Inst. Cajal Invest. biol.*
Trabajos del Instituto nacional de ciencias médicas. Madrid	*Trab. Inst. nac. Cienc. méd., Madr.*
Trabajos del Laboratorio de investigaciones biológicas de la Universidad de Madrid	*Trab. Lab. Invest. biol. Univ. Madr.*
Transactions of the Association of American Physicians	*Trans. Assoc. Amer. Physic.*
Transactions of the Edinburgh Obstetrical Society	*Trans. Edinb. Obstet. Soc.*
Transactions of the Faraday Society	*Trans. Faraday Soc.*
Transactions of the Ophthalmological Society of the U.K.	*Trans. ophthal. Soc. U.K.*
Transactions of the Optical Society	*Trans. opt. Soc., Lond.*
Transactions of the Royal Society of Canada	*Trans. Roy. Soc. Can.*
Transactions of the Royal Society of Edinburgh	*Trans. Roy. Soc. Edinb.*
Transactions of the Royal Society of South Africa	*Trans. Roy. Soc. S. Afr.*
Transactions of the Royal Society of Tropical Medicine and Hygiene	*Trans. R. soc. trop. Med. Hyg.*
Transactions of the Wisconsin Academy of Sciences, Arts and Letters	*Trans. Wis. Acad. Sci. Arts Lett.*
Triangel	*Triangel*

PERIODICAL	ABBREVIATION
Ugeskrift for Læger	*Ugeskr. Læg.*
United States Naval Medical Bulletin	*U.S. nav. med. Bull.*
University of California Publications	*Univ. Calif. Publ.*
Verhandelingen der (K) Akademie van Wetenschappen	*Verh. Akad. Wet., Amst.*
Verhandlungen der Deutschen Zoologischen Gesellschaft	*Verh. dtsch. zool. Ges.*
Verhandlungen der Gesellschaft Deutscher Naturforscher und Ärzte	*Verh. Ges. dtsch. Naturf. Ärzt.*
Verhandlungen des Naturhistorisch-medizinischen Vereins zu Heidelberg	*Verh. naturh.-med. Ver. Heidelb.*
Verhandlungen des Vereins der Schweizer Physiologen	*Verh. Ver. schweiz. Physiol.*
Verslagen van de gewone vergadering der wis- en natuurkundige afdeeling. Konink. Akademie van wetenschappen te Amsterdam	*Versl. gewone Vergad. Akad. Amst.*
Veterinary Record	*Vet. Rec.*
Virchows Archiv für pathologische Anatomie und Physiologie	*Virchows Arch.*
Vitamine und Hormone. Leipzig	*Vitam. u. Horm.*
Vitamins and Hormones. New York	*Vitam. & Horm.*
War Medicine	*War Med.*
Wiener Archiv für innere Medizin	*Wien. Arch. inn. Med.*
Wiener klinische Wochenschrift	*Wien. klin. Wschr.*
Wiener medizinische Wochenschrift	*Wien. med. Wschr.*
Wissenschaftliche Veröffentlichungen aus d. Siemens-Konzern	*Wiss. Veröff. Siemens*
Yale Journal of Biology and Medicine	*Yale J. Biol. Med.*
Yale Psychological Studies	*Yale psychol. Stud.*
Zeitschrift für allgemeine Physiologie	*Z. allg. Physiol.*
Zeitschrift für Augenheilkunde	*Z. Augenheilk.*
Zeitschrift für Biologie	*Z. Biol.*
Zeitschrift für die gesamte Anatomie	*Z. ges. Anat.* 1. *Z. Anat. EntwGesch.* 2. *Z. KonstLehre* 3. *Ergebn. Anat. EntwGesch.*
Zeitschrift für die gesamte experimentelle Medizin	*Z. ges. exp. Med.*
Zeitschrift für die gesamte Neurologie und Psychiatrie	*Z. ges. Neurol. Psychiat.*
Zeitschrift für experimentelle Pathologie und Therapie	*Z. exp. Path. Ther.*
Zeitschrift für Geburtshilfe und Gynäkologie	*Z. Geburtsh. Gynäk.*
Zeitschrift für Hals-, Nasen- und Ohrenheilkunde	*Z. Hals-, Nas.- u. Ohrenheilk.*
Zeitschrift für Hygiene und Infektionskrankheiten	*Z. Hyg. InfektKr.*
Zeitschrift für Immunitätsforschung	*Z. ImmunForsch.*
Zeitschrift für Kinderheilkunde	*Z. Kinderheilk.*
Zeitschrift für klinische Medizin	*Z. klin. Med.*
Zeitschrift für Kreislaufforschung	*Z. KreislForsch.*
Zeitschrift für mikroscopisch-anatomische Forschung	*Z. mikr.-anat. Forsch.*
Zeitschrift für Naturforschung	*Z. Naturf.*
Zeitschrift für physikalische Chemie	*Z. phys. Chem.*
Zeitschrift für physikalische und diätetische Therapie	*Z. phys. diätet. Ther.*
Zeitschrift für physiologische Chemie	*Hoppe-Seyl. Z.*
Zeitschrift für Psychologie und Physiologie der Sinnesorgane	*Z. Psychol. Physiol. Sinnesorg.*
Zeitschrift für Sinnesphysiologie	*Z. Sinnesphysiol.*
Zeitschrift für urologische Chirurgie	*Z. urol. Chir.*
Zeitschrift für vergleichende Physiologie	*Z. vergl. Physiol.*
Zeitschrift für Vitaminforschung	*Z. Vitaminforsch.*
Zeitschrift für wissenschaftliche Mikroskopie und für mikroskopische Technik	*Z. wiss. Mikr.*
Zeitschrift für wissenschaftliche Zoologie	*Z. wiss. Zool.*
Zeitschrift für Zellforschung und mikroskopische Anatomie	*Z. Zellforsch.*
Zentralblatt für allgemeine Pathologie und pathologische Anatomie	*Zbl. allg. Path. path. Anat.*
Zentralblatt für Bakteriologie, Parasitenkunde u. Infektionskrankheiten (Abteilung 1, Originale *or* Referate)	*Zbl. Bakt.* (*1. Abt. Orig.* or *Ref.*)
Zentralblatt für Bakteriologie, Parasitenkunde u. Infektionskrankheiten (Abteilung 2)	*Zbl. Bakt.* (*2. Abt.*)

Zentralblatt für Biochemie und Biophysik	*Zbl. Biochem. Biophys.*
Zentralblatt für die gesamte Gynäkologie und Geburtshilfe (Berlin)	*Zbl. ges. Gynäk.*
Zentralblatt für die medizinische Wissenschaften	*Zbl. med. Wiss.*
Zentralblatt für gesamte Physiologie und Pathologie des Stoffwechsels	*Zbl. ges. Physiol. Path. Stoffw.*
Zentralblatt für Gynäkologie (Leipzig)	*Zbl. Gynäk*
Zentralblatt für Physiologie	*Zbl. Physiol.*
Zentralblatt für Veterinärmedizin	*Zbl. Veterinärmed.*
Zoological Record	*Zool. Rec.*
Zoologische Jahrbücher	*Zool. Jb.*
Zoologiska Bidrag från Uppsala	*Zool. Bidr. Uppsala*

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PASSAGE OF CIRCULATING ADRENALINE INTO PERFUSED CEREBRAL VENTRICLES AND SUBARACHNOIDAL SPACE

By M. DRAŠKOCI,* W. FELDBERG and P. S. R. K. HARANATH*
From the National Institute for Medical Research, Mill Hill, London, N.W. 7

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In dogs, cats and mice injections of adrenaline into the cisterna or into the cerebral lateral ventricle produce changes in behaviour resembling a condition of anaesthesia (Bass, 1914; Leimdorfer & Metzner, 1949; Feldberg & Sherwood, 1954; Haley & McCormick, 1957; Reitter, 1957), but it is not known whether adrenaline can pass from the blood stream into the cerebral fluid of the cerebral ventricles and of the subarachnoidal space. In the present experiments this problem has been examined by infusing adrenaline intravenously whilst these spaces were perfused with artificial salt solution. The perfusate was then collected and assayed for adrenaline by the depression it produces on the acetylcholine contractions of the isolated rat uterus.

When assaying for adrenaline it was found that in larger doses adrenaline had a stimulating action on the rat uterus. This finding as well as details about the depressant action of adrenaline on the rat uterus are included in this paper.

So far little is known about the passage of pharmacologically active substances from the blood stream into the cerebrospinal fluid. Leimdorfer, Arana & Hack (1947) did not detect any adrenaline in the c.s.f. collected from the cisterna of cats 15 min after an intravenous infusion of 250 μg/kg. Paton (1953) found traces of hexamethonium in the c.s.f. when the drug had been injected into the blood stream. With a plasma concentration of about 100 μg/ml. the concentration of hexamethonium in the c.s.f. remained below 1 μg/ml. Mahfouz (1949) injected intravenously into an unanaesthetized patient tubocurarine 0·2 mg/kg. About half an hour later, when the plasma concentration had fallen to a level of about 1 μg/ml., the c.s.f. collected by lumbar puncture contained 2·5 μg/ml. On the other hand, Bhattacharya & Feldberg (1958*b*) could not detect any passage of tubocurarine into the c.s.f. of anaesthetized cats. They perfused from a cannula in the lateral ventricle the ventricular and the subarachnoidal space with Locke's solution, and injected 1–10 mg tubocurarine intravenously.

* W.H.O. Fellows.

No tubocurarine was detected in the cisternal effluent. With the same method they could show that the anticholinesterases eserine and neostigmine injected intravenously appeared in the cisternal effluent. Eserine passed more readily into the perfusion fluid than neostigmine and part of this passage occurred in the ventricles, part in the subarachnoidal space.

METHODS

Animals. The experiments were carried out in cats anaesthetized by intravenous chloralose (80 mg/kg) after preliminary ethyl chloride. A few days before the actual experiment, a Collison cannula was implanted aseptically under pentobarbitone anaesthesia into the lateral ventricle, according to the method described by Feldberg & Sherwood (1953). The method of perfusion from the lateral ventricle to the cisterna, and from the lateral ventricle to the aqueduct, was that described by Bhattacharya & Feldberg (1957, 1958*a*). In order to perfuse areas of the subarachnoidal space without the cerebral ventricles, two procedures were adopted, perfusion from the epicranial subarachnoidal space to the cisterna and perfusion from the cisterna to the lumbar space or *vice versa*. The cannula used for perfusion

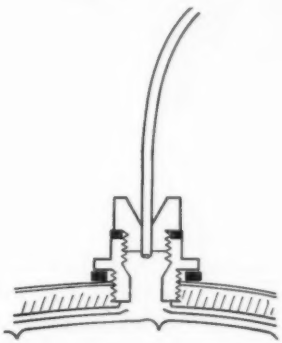

Fig. 1. Diagram of the cannula used for perfusion from the epicranial subarachnoidal space.

from the epicranial subarachnoidal space is diagrammatically represented in Fig. 1. It consists of two parts, an outer sleeve which is screwed into the skull, and an inner part, the actual cannula, which is taken by the inner thread of the sleeve. To avoid the rim of the sleeve pressing the dura against the brain tissue, thereby preventing the free passage of fluid, a rubber washer (indicated in black in the diagram) is inserted between the outer flange of the sleeve and the skull. Another thin washer is placed between the sleeve and the actual cannula. A polythene tube is firmly fixed into the centre of the cannula and can be connected with the perfusion system. During perfusion from the epicranial subarachnoidal space, the cat was lying on its back and the head was raised and flexed forward so that the occipital surface of the skull was horizontal. When perfusion was carried out from the cisterna to the lumbar space or *vice versa*, the cat was lying on its side and a cannula was tied into the lumbar subarachnoidal space.

Mechanical arrangements. The perfusion fluid in all perfusion experiments was Locke's solution, containing ascorbic acid 1 μg/ml. to prevent the destruction of adrenaline. The rate of perfusion was 0·1 ml./min and the effluent was usually collected in 20 min samples. In

order to prevent destruction of adrenaline during the period until the sample was tested, the 20 min samples were collected in 0·1 ml. N/10-HCl.

A Palmer slow infusion pump, set to deliver 0·2 ml./min, was used to infuse adrenaline solution at a rate of 40 μg/kg/min into the right femoral vein. The entrance of adrenaline into the circulation was checked by recording the arterial blood pressure in the left femoral artery. Artificial ventilation was applied through a cannula in the trachea throughout the experiment.

Arterial samples of 2 ml. blood were taken from the right femoral artery at hourly intervals with a syringe containing small amounts of heparin. The samples were immediately centrifuged, the plasma was separated and kept in cold storage until the assay was performed. All assays were performed on the same day. The values of adrenaline refer to the base.

Assay of adrenaline. Perfusate and plasma were assayed for adrenaline on the acetylcholine contractions of the rat uterus, according to the method described by de Jalon (de Jalon, Bayo & de Jalon, 1945). The uteri were taken from virgin rats weighing about 200 g; the rats were either in oestrus or brought into this condition by subcutaneous injection of stilboestrol 100 μg/100 g the day before the experiment. The uteri were suspended in 15 ml. of a salt solution used by Gaddum, Peart & Vogt (1949). When the adrenaline content of plasma was tested, lysergic acid diethylamide 5 μg/l. of salt solution was added, according to the procedure of Franko, Bragg & Watts (1957), in order to antagonize the effect of any 5-HT which might be present in the plasma.

RESULTS

Effect on rat uterus of adrenaline, of c.s.f., cisternal effluent, or plasma, collected before intravenous infusion of adrenaline

Adrenaline. It was usually possible to detect on the rat uterus 5 ng (sometimes not less than 20 ng and occasionally as little as 1 ng) of adrenaline by the depression of the acetylcholine contractions. Usually the maximal depression occurred with the acetylcholine contraction elicited whilst the adrenaline was in the bath or immediately after it was washed out. But in several instances, particularly when the rat had not been treated with stilboestrol, the depressant effect was delayed and was maximal with the subsequent contraction elicited 2 min later. Such instances are illustrated in Fig. 2. The contractions shown at *A* were from a sensitive preparation which responded to 2 ng adrenaline but the depression did not occur with the first acetylcholine contraction whilst the adrenaline was in the bath, but only with the subsequent acetylcholine contraction. When the dose of adrenaline was increased to 3 ng, the depression took place with the first acetylcholine contraction. We have frequently observed this difference between smaller and larger doses. In addition, it was found that the same dose of adrenaline might at some time in the course of an experiment depress maximally the first, and at another time the second acetylcholine contraction. Such a shift happened, for instance, in the experiment of Fig. 2*B* with 10 ng adrenaline. First its maximal depressant effect occurred immediately after the adrenaline was washed

out. Later, when the sensitivity of the preparation had somewhat increased, the main depressant effect was delayed and occurred with the second contraction.

Adrenaline was found to have in addition a stimulating action on the rat uterus when given in a larger dose (500 ng). Such a stimulating effect has been observed on the guinea-pig uterus by Balassa (1941) and by Singh & Singh (1951). Balassa, however, pointed out that adrenaline lacked this stimulating effect on the rat uterus, whereas Bonney & Ferguson (1950) obtained it occasionally on the uterus of this species.

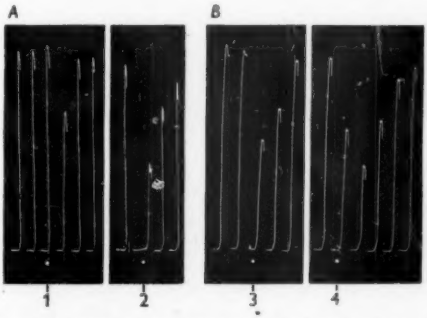

Fig. 2. Effect of small doses of adrenaline on the contractions to 1 μg of acetylcholine of the rat uterus suspended in a 15 ml. bath. *A* and *B*, different preparations. Acetylcholine given every 2 min and left in the bath for 25 sec. The contractions marked by a dot were obtained immediately after washing out a small dose of adrenaline which had been in the bath for 30 sec. The dose of adrenaline was (1) 2 ng, (2) 3 ng and (3 and 4) 10 ng. (For details see text.)

In the experiment of Fig. 3*A*, the contraction produced by 500 ng adrenaline is nearly as great as that produced by 1 μg acetylcholine. When the adrenaline is washed out, the preparation is for a short time practically insensitive to 1 μg acetylcholine. This is shown in Fig. 3*B*. The stimulating effect of adrenaline decreases progressively with repeated application at short intervals, as is shown at 4–8 in Fig. 3*A*. This finding suggests that adrenaline depresses its own stimulating effect. When 100 or 200 ng of adrenaline is given together with 1 μg of acetylcholine, the ensuing contraction may be equal to that produced by 1 μg acetylcholine alone or be slightly depressed only; but a depressant effect occurs after the adrenaline is washed out when a subsequent dose of 1 μg acetylcholine is given alone (Fig. 3*A*).

Cerebrospinal fluid collected from the cisterna before the perfusion of the ventricular spaces and the intravenous infusion of adrenaline had begun had no stimulating effect of its own on the rat uterus, and usually had no influence on the acetylcholine contractions; in some experiments it

increased the acetylcholine contractions. This sensitizing effect was either transient (Fig. 4*C*) or affected, though in diminishing degree, two or three acetylcholine contractions which followed each other at 2 min intervals (Fig. 4*D*).

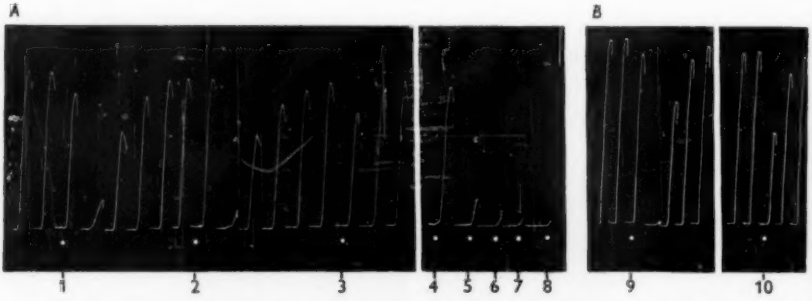

Fig. 3. Effect of large doses of adrenaline on the rat uterus suspended in a 15 ml. bath. *A* and *B*, different preparations. The unmarked contractions are due to 1 μg ACh given at 2 min intervals and left in the bath for 25 sec. The contractions marked by a white dot are due either to 500 ng adrenaline alone (4, 5, 6, 7, 8 and 9) or to 1 μg ACh given together with adrenaline 100 ng (1), 200 ng (2) and 20 ng (3). The contraction at 10 was obtained by giving 1 μg ACh immediately after washing out 7 ng adrenaline left in the bath for 30 sec. (For details see text.)

In six experiments definite adrenaline-like effects were obtained. In one, a sample of 1·9 ml. c.s.f. was collected, and the effect of 1 ml., shown at Fig. 4*A*, corresponded to 40 ng adrenaline; the blood collected before the adrenaline infusion contained an adrenaline-like substance corresponding to 250 ng/ml. plasma. In another experiment the c.s.f. was collected in two successive samples of 1·2 ml. each, but an adrenaline-like effect corresponding to adrenaline 8 ng/ml. was obtained with the second sample only. In order to find out whether c.s.f. obtained from different parts of the subarachnoidal space behaved differently, the c.s.f., in later experiments, was collected in three successive samples, lowering the level of the opening of the cisternal cannula with each sample so as to increase the draining from the spinal subarachnoidal space. During the taking of the first sample the level of the opening was at the occipito-atlantic membrane, during the second at the height of the spinal cord and during that of the third below this level; in addition, the hind quarters were lifted during the collection of the last sample. The results are given in Table 1. They are not uniform in so far as the first sample was free from detectable amounts of the adrenaline-like substance twice only, and in only three out of four did the second sample yield the highest value. Part of the assay of the samples from Expt. 1 of Table 1 is shown in Fig. 4 at *B*.

Cisternal effluent. The control perfusate collected for 30–60 min before the adrenaline infusion was tested on the rat uterus in amounts of up to 1 ml. In six experiments it did not influence the acetylcholine contractions, in nine experiments it potentiated the contractions, in eight it

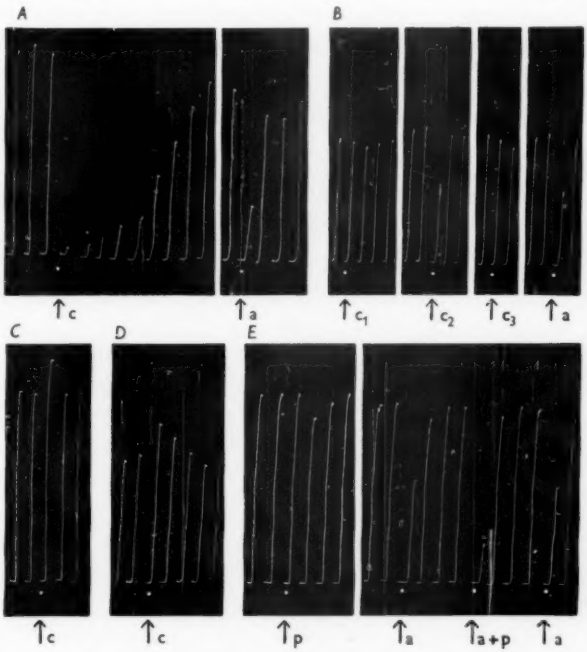

Fig. 4. Effect of c.s.f., adrenaline and cisternal perfusate on the contractions produced by 1 μg ACh on the rat uterus suspended in a 15 ml. bath. *A–E*, five different preparations. The ACh was given every 2 min and left in the bath for a time, kept constant for each uterus, which varied between 20 and 40 sec. The contractions marked by a white spot were obtained immediately after washing out either c.s.f. (c), adrenaline (a) or perfusate (p), which had been kept in the bath for 30 sec. The tests for c, a and p were as follows. (*A*) at c, 1 ml. c.s.f.; at a, 10 ng adrenaline. (*B*) successive samples of c.s.f.; at c_1, 0·5 ml. of first sample, at c_2, 0·25 ml. of second, and at c_3, 0·5 ml. of third; at a, 3 ng adrenaline. (*C*) at c, 0·4 ml. c.s.f. (*D*) at c, 0·6 ml. c.s.f. (*E*) at p, 1 ml. perfusate, at a, 5 ng adrenaline, at a + p, 5 ng of adrenaline together with 1 ml. perfusate. (For details see text.)

slightly reduced them, and in two it produced a depression corresponding to adrenaline 10 ng/ml. The c.s.f. of one of these two experiments had produced an adrenaline-like effect corresponding to adrenaline 40 ng/ml.; this is the experiment of Fig. 4*A*. In the other experiments in which adrenaline-like effects had been obtained with c.s.f. no measurable amount of an adrenaline-like substance was detected in the cisternal perfusate.

In those eight experiments in which there was slight reduction of the acetylcholine contraction with perfusate, the reduction sometimes affected the second contraction after washing out the perfusate, although adrenaline itself, even if given in small doses, reduced the first contraction. When such perfusate, however, was tested together with a small amount of adrenaline, it enhanced its immediate depressant effect. This is illustrated in Fig. 4*E*.

TABLE 1. Assay of adrenaline-like activity on rat uterus of successive samples of c.s.f. collected from the cisterna

Expt.	Volume of three successive samples of c.s.f. (ml.)	Activity expressed as adrenaline ng/ml.
1	1·0	0
	0·8	10
	1·0	0
2	0·6	0
	0·6	10
	0·6	5
3	0·8	17
	1·1	20
	1·2	14
4	0·6	50
	0·9	12
	1·1	13

Plasma. When tested in amounts of 0·1 ml. or less on the rat uterus treated with lysergic acid, plasma usually either had no effect or caused a small potentiation of the acetylcholine contractions. In a few experiments the plasma produced small adrenaline-like effects and in one experiment the adrenaline-like effect was pronounced and corresponded to that of adrenaline 250 ng/ml. Mention of this experiment has been made when describing the effect of c.s.f. on the rat uterus. The amounts used for assay of plasma obtained during the adrenaline infusion, however, were so small (0·002–0·0005 ml.) that interference of the assay by the potentiating effect of plasma was unlikely.

Arterial blood pressure and plasma adrenaline during intravenous infusion of adrenaline

Arterial blood pressure. The rise in arterial blood pressure which occurs when the intravenous infusion of adrenaline 40 μg/kg/min is begun is not maintained even though the infusion is continued. After 5–20 min the pressure begins to fall gradually, reaches the original level within 1–2 hr and then continues to fall, sometimes to very low levels.

Plasma adrenaline. The plasma level of adrenaline during its intravenous infusion at a rate of 40 μg/kg/min was found in different cats to vary

greatly between less than 1 μg and more than 30 μg/ml., usually between 4 and 10 μg/ml. In the same cat, however, approximately the same level was often maintained throughout the experiment, independent of whether the level was high or low. Such a result suggests that once a given plasma level was reached the amounts of adrenaline eliminated from the blood must have been about equal to those infused. In a number of experiments

TABLE 2. Plasma concentration of adrenaline during its intravenous infusion at a rate of 40 μg/kg/min

Expt. no.	Weight of cat (kg)	Adrenaline (μg/ml. of successive hourly samples) 1st hr	2nd hr	3rd hr	4th hr	5th hr
1*	2·2	1·7	0·7	1·3	—	—
2	2·7	1·8	2·2	—	—	—
3	3·1	3·1	3·3	4·0	—	—
4	2·1	3·6	3·3	2·8	2·5	—
5	1·6	3·7	3·3	—	2·4	—
6	2·7	4·3	4·3	—	—	—
7	2·3	5·0	4·3	3·7	3·7	—
8*	2·4	5·7	5·7	5·7	5·7	—
9	1·9	5·7	6·7	6·1	—	—
10	2·2	6·0	4·0	3·3	3·3	—
11	1·9	6·0	8·5	6·0	—	8·0
12	2·1	6·3	5·9	6·7	6·7	—
13*	2·3	6·7	6·0	5·0	5·7	—
14	2·4	6·7	6·7	6·7	6·7	—
15**	1·9	7·5	5·0	4·5	6·0	6·0
16	1·7	7·5	17·1	24·0	—	—
17	3·0	8·0	8·3	7·7	7·1	—
18	2·8	—	8·3	10·0	—	—
19	3·0	8·5	6·7	—	—	—
20	2·5	10·0	8·3	6·7	—	—
21***	2·0	10·0	2·5	—	—	—
22	2·6	11·4	8·9	5·9	8·0	—
23	2·2	11·4	9·5	9·4	8·4	11·1
24	3·4	12·5	15·4	16·0	11·1	—
25**	3·3	14·3	15·4	8·0	9·8	—
26	2·9	20·0	11·0	8·0	17·0	—
27	3·1	20·0	11·7	8·7	10·0	—
28	2·6	20·0	32·0	25·0	—	—

(*), some lung oedema; (**) lung oedema; (***) severe lung oedema.

the adrenaline level in the successive hourly samples of plasma either fell or rose or showed fluctuations. This is shown in Table 2, which summarizes the results of the plasma–adrenaline assays of all experiments.

There was no definite correlation between the plasma level of adrenaline reached on continuous infusion and the weight of the cat, although high plasma levels were more frequently found in the larger animals, in which the absolute amounts of adrenaline infused per minute were greater than in the smaller cats. It is known that continuous intravenous infusion of adrenaline is likely to produce lung oedema; this happened also in several of our cats (see Table 2). The oedema became evident in the course of an experiment by the fact that in spite of strong artificial ventilation the

arterial blood samples were no longer fully oxygenated; the oedema was confirmed post-mortem. Since the oedema occurred with both low and high levels of plasma adrenaline, pulmonary oedema does not explain the observed individual variations.

The appearance of adrenaline in effluent from perfused subarachnoidal and ventricular spaces during intravenous infusion of adrenaline

With all the procedures of perfusion adopted adrenaline appeared in the effluent during its intravenous infusion. This finding showed that adrenaline enters the perfusion fluid in the subarachnoidal space as well as in the cerebral ventricles. The adrenaline output was never as high when perfusion was effected from the lateral ventricle to the aqueduct as it was in some of the experiments in which perfusion was from the lateral ventricle to the cisterna. The high output when it occurred in the cisternal effluent must, therefore, have originated from the subarachnoidal space. This conclusion is supported by the finding that perfusion from the epicerebral subarachnoidal space to the cisterna often yielded a high adrenaline output in the cisternal effluent. A high output was also obtained when the spinal subarachnoidal space was perfused, which suggests that there is no significant difference in the escape of adrenaline from blood into the subarachnoidal space of the brain and of the spinal cord. In detail the results were as follows.

Perfusion from lateral ventricle to cisterna. During its intravenous infusion adrenaline was always detected in the cisternal effluent, but the amounts varied from one experiment to another independently of the plasma concentration and of the body weight of the cats. This is evident from the results shown in Table 3.

From the values given in the first two columns of Table 3 it is seen that in several experiments the cisternal effluent collected during the first 15 or 30 min of adrenaline infusion contained either no detectable amounts of adrenaline or traces only. During this period the maximal rise occurred in the arterial blood pressure. The adrenaline output obtained during the subsequent half-hour periods did not show a regular course. In Expts. nos. 17, 4, 10, and 11 of Table 3, the highest value was obtained in the second half-hour period, in the others in later periods. The values could remain relatively constant from sample to sample or might show great variation; particularly high values were not maintained.

Perfusion from lateral ventricle to aqueduct. Adrenaline appeared in the aqueductal effluent during its intravenous infusion. In several experiments the outflow was first collected for 2–3 hr from the cisterna and then for another 1–1½ hr from the aqueduct. The change over to the aqueductal cannula did not require more than 30 min; during this time the inflow was

continued but the effluent was not collected. When the output of adrenaline in the cisternal effluent had been low it was not affected by the change over (Expts. nos. 11 and 23 of Table 3) but when it had been high it fell to a low value (Expts. nos. 4, 10 and 14 of Table 3).

TABLE 3. Adrenaline output in cisternal or aqueductal (italic figures) effluent during intravenous infusion of adrenaline 40 μg/kg/min. Inflow from cannula implanted in lateral ventricle. Outflow in group *A* throughout from cisterna; in group *B* first from cisterna, then from aqueduct, and in group C throughout from aqueduct. Numbers of experiments are as those of Table 2

Group	Expt. no.	Adrenaline output (ng/min) in successive 30 min samples									
Minutes ...		1–30*	30–60	60–90	90–120	120–150	150–180	180–210	210–240	240–270	270–300
	1	(0) Traces	2·2	2·2	2·0	2·3	3·1	—	—	—	—
	5	Traces	2·0	3·1	3·5	—	—	5·8	—	—	—
	12	(0·3) 7·3	24·7	43·3	14·3	21·3	20·3	23·5	39·3	—	—
A	13	(0) Traces	1·3	3·2	4·3	6·3	5·2	3·7	4·3		
	17	(1·5) 4·7	8·1	4·9	2·8	2·1	2·3	2·6	3·3	—	—
	21	1·8	5·4	12·5	16·2	—	—	—	—	—	—
	27	(0·85) 4·4	6·3	10·2	5·9	6·0	4·8	3·1	—	—	—
	4	13·5	63·3	22·2	11·8	—	*1·7*	*2·2*	*1·6*	—	—
	10	70·0	208	32·0	15·0	—	*3·0*	*2·3*	*1·8*	—	—
B	11	(1·4) 1·9	3·9	2·1	1·4	1·2	1·5	—	*1·4*	*1·2*	—
	14	4·0	17·6	31·4	14·7	—	*2·7*	*2·1*	*2·7*	—	—
	23	(0) Traces	1·3	4·8	2·8	5·3	3·6	—	*2·5*	*5·0*	*5·0*
	16	*1·4*	*5·5*	*5·8*	*6·9*	*6·9*	*7·7*	*7·7*	—	—	—
C	22	*4·1*	*7·6*	—	*2·8*	*3·1*	*2·6*	*1·5*	—	—	—
	24	*1·0*	*3·5*	*3·6*	*4·2*	*4·3*	*6·0*	*7·3*	*6·9*	—	—

* When two values are given, the one in brackets refers to the first 15 min, the one without brackets to the second 15 min period.

In three experiments, in which the outflow was collected throughout from the aqueduct, the adrenaline output was low from the beginning to the end of perfusion. The maximum output was never higher than 7·7 ng/min. In two of these experiments the output increased and in one it decreased during the course of the intravenous infusion of adrenaline. There was some but no definite correlation between the adrenaline output and the level of plasma adrenaline.

Perfusion from the epicerebral subarachnoidal space to cisterna. With this method of perfusion the outflow was usually less than the inflow; nevertheless the output of adrenaline during its intravenous infusion was high. The best outflow for prolonged periods was obtained when the cat was lying on its back with the head raised and flexed forward. The results of two such experiments are given in Table 4.

At the end of these experiments dye was perfused for 10 min, and post-mortem the dura was found to be intensely stained over the cerebral hemisphere of the side where the cannula was implanted, whereas over the opposite cerebral hemisphere only a few small areas of the dura were stained. There was scarcely any staining over the dorsal surface of the cerebellum but there was intense staining of the surface of the superior and inferior colliculi, of the ventral surface of the brain stem, pons and

TABLE 4. Adrenaline output in cisternal effluent during intravenous infusion of 40 μg/kg/min with inflow from cannula implanted into the right epicerebral subarachnoidal space. Numbers of experiments are as those of Table 2

Minutes of perfusion	Expt. 2		Expt. 20	
	Volume (ml.)	Adrenaline (ng/min)	Volume (ml.)	Adrenaline (ng/min)
1– 10	1·3	2·6	2·4	15
10– 30	1·9	28·5		
30– 60	2·7	40·0	2·3	42
60– 90	2·3	33·0	2·4	29
90–120	2·4	53·0	2·3	34
120–150	2·7	44·0	2·2	26
150–180	—	—	2·4	29
180–210	—	—	2·2	26

medulla oblongata. The base of the skull was also heavily stained. There was no staining of the floor of the fourth ventricle. These findings suggest that the perfused fluid flowed mainly over the ipsilateral cerebral hemisphere and then passed along the tentorium and base of the brain stem on to the cisterna.

In three experiments in which the cat was lying on its belly in the usual way, with the head raised and neck slightly flexed, perfusion was started from the epicerebral subarachnoid space to the cisterna, but was later continued from the lateral ventricle to either the cisterna or the aqueduct. In one of these experiments the output of adrenaline during perfusion from the epicerebral subarachnoidal space was over 100 ng/min; it fell to 8 ng/min when the route of perfusion was from the lateral ventricle to the aqueduct. In the two other experiments the adrenaline output during perfusion from the epicerebral subarachnoidal spaces showed variation between 55 and 24 ng/min, and fell in both to 16 ng/min during subsequent perfusion from the lateral ventricle to the cisterna. When the cisternal cannula was finally replaced by an aqueductal cannula the adrenaline output fell to 6·6 ng/min in the one and to 1·5 ng/min in the other experiment.

Perfusion of the spinal subarachnoidal space. Whether the route of perfusion was from the cisterna to the lumbar space or vice versa, the outflow was often less than the inflow. When adrenaline was infused intravenously during such a perfusion it appeared in the effluent. This is shown

in Table 5. In the first experiment of this table perfusion was effected from the lumbar space to the cisterna, in the third experiment it was in the opposite direction. In the second experiment the first two 15 min samples were collected from the lumbar space; then the direction of perfusion was reversed and the later samples were collected from the cisterna. In all

TABLE 5. Adrenaline output (ng/min) from the perfused spinal subarachnoidal space during intravenous infusion of 40 μg/kg/min. Numbers of experiments are as those of Table 2

Minutes of perfusion	Expt. 3	Expt. 18	Expt. 9
1– 15	↑ 1·5	↓ 0·8	0
15– 30	1·7	22·5	0·7
30– 60	5·4		6·0
60– 90	8·4		14·0
90–120	11·7	↑ 34	26·0
120–150	34·0	117	26·0
150–180	44·0	138	↓ 26·0
180–210		139	

The direction of the arrows indicates whether perfusion is from lumbar space to cisterna (↑) or vice versa (↓).

three experiments the output became high in the course of the infusion and corresponded to values obtained in experiments in which the cranial subarachnoidal space was perfused or in which the direction of perfusion was from the lateral ventricle to the cisterna, including part of the subarachnoidal space.

Appearance in c.s.f. of adrenaline during its intravenous infusion

In one experiment adrenaline 40 μg/kg/min was infused intravenously for 2 hr; 1 ml. c.s.f. was then removed from the cisterna and 15 min later another 0·6 ml. from the lumbar space. Both samples were assayed on the rat uterus treated with lysergic acid. The assay of the cisternal sample yielded 1·7 μg, that of the lumbar sample 1·3 μg adrenaline/ml.

DISCUSSION

The present experiments show that adrenaline can pass from the blood stream into the perfused cerebral ventricles and the subarachnoidal space surrounding the brain and spinal cord. This finding agrees with observations by Wallace & Brodie (1940) that intravenous bromide and iodide enter the c.s.f. in the ventricular cavities as well as in the epicerebral and lumbar subarachnoidal space.

Not all experiments in which the rate of entrance into these different compartments has been compared were done under conditions which actually allowed such comparisons to be made. It seems, however, clear that different substances behave differently. For instance, the passage

of labelled sodium and penicillin into the ventricular spaces appears to be greater than into the subarachnoidal space (Dumoff-Stanley, Dowling & Sweet, 1946; Sweet, Selverstone, Solomon & Backay, 1949), whereas the reverse is true for nitrous oxide (Bessman, Alman, Hayes & Fazekas, 1952). Labelled albumin also was found in greater concentration in the c.s.f. of the spinal subarachnoidal space than in that collected from any other part. These experiments were, however, done on hydrocephalic children (Fishman & Ransohoff, 1955). In our experiments more adrenaline appeared in the effluent from the perfused subarachnoidal space than in that from the cerebral ventricles.

The choroid plexus cannot be the site of passage for the adrenaline entering the subarachnoidal space but it could be the site for entering the cerebral ventricles. We do not know if the adrenaline in the subarachnoidal space originates from the meningeal vessels and passes from these directly into the subarachnoidal space, or whether it passes first from the blood capillaries into the intra- or extracellular fluid of the brain.

That diffusion can occur from the brain tissue into the cerebral ventricular and subarachnoidal space has been shown for acetylcholine. Provided its destruction is prevented by an anticholinesterase, acetylcholine appears in the subarachnoidal space as well as in the cerebral ventricles (MacIntosh & Oborin, 1953; Burns & MacIntosh, 1955; Bhattacharya & Feldberg, 1958*b*). As this acetylcholine is not derived from the blood but released from the nervous tissue these results cannot be directly applied to the present ones with adrenaline. However, the following findings would be compatible with the idea that adrenaline does not pass directly from the blood vessels into the subarachnoidal space, but passes first into the tissue spaces of the brain or spinal cord. There is little or no passage of adrenaline into the subarachnoidal space during the initial period of intravenous adrenaline infusion whilst the arterial blood pressure is high; there is always a delay, the maximal output of adrenaline occurring only after intravenous infusion for 30–60 min. There is a lack of quantitative relationship between output of adrenaline and plasma-adrenaline levels.

The amounts of adrenaline used for intravenous infusion were greater than would usually be released into the circulation, and the question therefore arises whether adrenaline released under physiological conditions occurs in detectable amounts in the c.s.f. The finding that c.s.f. or perfusate, when collected before an intravenous infusion of adrenaline was begun, sometimes exerted an adrenaline-like effect on the rat uterus, could be taken as evidence for the passage of endogenous adrenaline from the blood into the c.s.f., although it is not certain whether the adrenaline originated from the blood stream. However, the fact that in one experiment at least the blood removed at the same time yielded a high adrenaline

equivalent makes it likely that the effect produced by the c.s.f. and perfusate, in the absence of artificially administered adrenaline, was also due to adrenaline itself which had been released into the circulation.

Minute amounts of adrenaline have also been found by Vogt (1954) in the c.s.f. of cats, although none had been administered. Its presence in human c.s.f. has been reported by Weil-Malherbe & Liddel (1954) and by Manger, Schwarz, Baars, Wakim, Bollman, Petersen & Berkson (1957).

Feldberg & Sherwood (1954) discussed the possibility that 'exhaustion' or 'fatigue' which follows strong emotional disturbances and other conditions associated with the sympatho-adrenal discharge described by Cannon (1929) in so-called emergency states results from the passage of circulating adrenaline into the c.s.f. of the ventricular cavities. In our experiments with exogenous adrenaline its passage into the ventricular spaces was less than into the subarachnoidal spaces. Nevertheless, the presence of even minute amounts of adrenaline in the ventricular fluid may suffice to produce a condition of languor. If this is so, then we would have to assume that the release of adrenaline into the blood stream would first produce heightened alertness by its action from the blood stream (Bonvallet, Dell & Hiebel, 1954; Rothballer, 1956) but later, after passing into the c.s.f., a condition of languor.

SUMMARY

1. In anaesthetized cats the cerebral ventricles and different parts of the subarachnoidal space were perfused with Locke's solution at a rate of 0·1 ml./min whilst adrenaline was infused intravenously at a rate of 40 μg/kg/min. Under these conditions adrenaline was found to enter the perfusate in the cerebral ventricles and even more in the subarachnoidal space, as is shown by the following results:

(*a*) When the direction of perfusion was from the lateral ventricle to the aqueduct, thus excluding the subarachnoidal space from the perfusion, the output of adrenaline in the aqueductal effluent was always small and varied between 1·2 and 7·7 ng/min.

(*b*) When the direction of perfusion was from the lateral ventricle to the cisterna, so that the ventricles and part of the subarachnoidal space were perfused, the output of adrenaline in the cisternal effluent varied greatly and sometimes rose to over 100 ng/min.

(*c*) When the direction of perfusion was from the epicerebral subarachnoidal space to the cisterna, thus excluding the cerebral ventricles from the perfusion, the output of adrenaline varied between 20 and over 100 ng/min.

(*d*) When the direction of perfusion was from the cisterna to the lumbar space, thus perfusing the spinal subarachnoidal space, the output of adrenaline also reached values of between 20 and over 100 ng/min.

2. On intravenous infusion of adrenaline into anaesthetized cats at a rate of 40 μg/kg/min the plasma adrenaline levels reached in different cats varied between less than 1 and more than 30 μg/ml., usually between 4 and 10 μg/ml. There was no direct relation between plasma-adrenaline levels and output of adrenaline into the perfused ventricular and subarachnoidal space.

3. The passage of adrenaline into the subarachnoidal space, and probably also into the ventricular cavities, occurs also when these spaces are not perfused with artificial salt solution, since adrenaline was found in the c.s.f. collected from the cisterna and lumbar space after a prolonged period of intravenous infusion of adrenaline 40 μg/kg/min.

4. Cerebrospinal fluid or perfusate collected from the cisterna before an intravenous infusion of adrenaline usually produced no adrenaline-like effect on the rat uterus, but in a few experiments such an effect was obtained; that of perfusate corresponded to up to 10 ng/ml. and that of c.s.f. in one experiment to adrenaline 40 ng/ml.

5. When assaying adrenaline by the depression it produced on the acetylcholine contraction of the rat uterus it was found that in larger doses it had a direct stimulating effect on the rat uterus.

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ABSORPTION OF HISTAMINE INTO THE BLOOD STREAM ON PERFUSION OF THE CEREBRAL VENTRICLES, AND ITS UPTAKE BY BRAIN TISSUE

By M. DRAŠKOCI,* W. FELDBERG, K. FLEISCHHAUER† AND P. S. R. K. HARANATH*

From the National Institute for Medical Research, Mill Hill, London, N.W. 7

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Bhawe (1958) has shown that when histamine is injected into the lateral cerebral ventricle of an anaesthetized cat acid gastric secretion occurs. This secretion was attributed to passage of histamine into the blood stream and it was assumed that the histamine had passed from the ventricles into the subarachnoid space and then into the endocranial venous sinuses. The present experiments show that histamine also passes into the blood stream from the cerebral ventricles without entering the subarachnoid space. In addition, it was found that histamine perfused through the cerebral ventricles entered the brain tissue surrounding the ventricular cavities and that this uptake of histamine showed differences dependent on the structures of the ventricular wall.

METHODS

The experiments were carried out in cats anaesthetized by intraperitoneal injection of pentobarbitone sodium 35 mg/kg; additional pentobarbitone was injected when it became necessary in the course of the experiment. The trachea was cannulated. Histamine was usually perfused through the lateral and third ventricles. The fourth ventricle was not included in the perfusion since, in order to prevent the perfusion fluid from entering into the subarachnoid space, the effluent was collected from a cannula inserted into the aqueduct, as described by Bhattacharya & Feldberg (1956). The effluent was assayed for histamine after its volume had been measured. The absorption of histamine into the blood stream was shown by estimating the acid gastric secretion.

Perfusion of cerebral ventricles. The fluid used for perfusion was that introduced by Merlis (1940) and later used by Leusen (1949). Its composition, which is approximately that of normal cerebrospinal fluid, is as follows (g/l.): NaCl 8·1; KCl 0·25; $CaCl_2$ 0·14; $MgCl_2$ 0·11; $NaHCO_3$ 1·76; NaH_2PO_4 0·07; $CO(NH_2)_2$ 0·13; and glucose 0·61. This solution will be referred to as artificial cerebrospinal fluid (artificial c.s.f.). A Collison cannula was implanted into the left lateral ventricle as described by Feldberg & Sherwood (1953). The perfusion was maintained by a continuous slow injector (C. F. Palmer, Ltd.) at a rate of 0·1 ml./min. In order to insert the outflow cannula into the aqueduct the muscle layers covering the atlanto-occipital membrane and the lower part of the occipital bone were dissected away, and the

* W.H.O. Fellow. † British Council Scholar.

membrane was opened. The lower part of the vermis of the cerebellum was exposed by nibbling away the margins of the occipital bone at the border of the foramen magnum. This made it possible to lift the cerebellum gently with a small curved spatula from the medulla oblongata and to insert a fine polythene tube along the floor of the fourth ventricle into the middle of the aqueduct. Perfusion was always begun with artificial c.s.f. without histamine and was continued for more than 1 hr before the ventricles were perfused with histamine 1:1000 or 1:10,000 at a rate of 0·1 ml./min.

Injections of histamine into the lateral ventricle and into the cisterna. In a few experiments the effect of an intraventricular injection of 500 μg histamine in 0·2 ml. volume on acid gastric secretion was examined under conditions which prevented the histamine from passing into the subarachnoid space. The ventricular cavities were first perfused for some time in the usual manner with artificial c.s.f. Perfusion was then stopped but the outflow was allowed to drain off from the aqueductal cannula; 0·2–0·3 ml. effluent was collected before the flow ceased. The aqueductal cannula was then closed by inserting a small glass stopper and the histamine was slowly injected through the ventricular cannula.

In order to find out whether histamine was absorbed from the subarachnoid space it was, in one experiment, infused into the cisterna under conditions which prevented its entrance into the cerebral ventricles. The cisterna was cannulated by puncturing the atlanto-occipital membrane and c.s.f. fluid (0·8 ml.) was allowed to flow out; the perfusion pump was then connected to the cisternal cannula through a polythene tube and a 1:1000 histamine solution was infused into the cisterna at a rate of 0·1 ml./min for 5½ min. The dead space of the cannula was about 0·05 ml. so that the histamine was in fact infused for 5 min only. The infusion was stopped, the cisternal cannula was closed and kept closed during the following 2 hr, during which acid gastric secretion was observed. In order to prevent any histamine from entering the cerebral ventricles, a counterflow of histamine-free fluid at half the rate was maintained from the implanted cannula during the 5½ min period of histamine infusion. The evidence that histamine had not entered the cerebral ventricles was obtained when, at the end of the experiment, the procedure was repeated, but with dye instead of histamine in the fluid used for cisternal infusion. Post mortem the outside surface and meninges of the spinal cord and the base of the brain stem and hypothalamus were found to be deeply stained but there was no staining in the fourth, third or lateral ventricles, nor of the surface of the cerebral hemispheres.

Acid gastric secretion was estimated by the method of Edkins (1906). The details of the procedure were essentially those described by Bhawe (1958). The cats were kept without food for 24 hr. A polythene tube was inserted into the stomach through a small cut on the antimesenteric border below the pyloro-duodenal junction, and after washing out the stomach it was filled with 15 ml. warm NaCl solution 0·9 % (w/v) introduced through the polythene tube and allowed to remain in the stomach for 20 min. The solution was then removed and replaced by another 15 ml. NaCl solution 0·9 %, and the procedure was repeated every 20 min. The volume of the 20 min samples was measured and their free HCl content titrated against 0·01 N-NaOH, with Töpfer's reagent as indicator. In several experiments the acid gastric secretion was compared with that produced by a slow infusion of histamine into the femoral vein through a continuous slow injector.

Histamine uptake by brain tissue

In order to determine the amounts of histamine taken up by various parts of the brain, samples of tissue were removed from different regions lining the ventricles and also from parts away from the perfused area. After perfusion of the cerebral ventricles with histamine 1:1000 for 1 hr and then with artificial c.s.f. for 20 min the cat was killed by an intravenous overdose of pentobarbitone sodium. The brain was quickly removed and cut in the mid line. Unless otherwise stated the tissue samples were taken from the left half of the brain, the side of cannulation of the lateral ventricle. Small blocks of tissue, usually weighing 20–40 mg

but sometimes as much as 70 mg, were cut out with fine scissors, dipped into a few millilitres of artificial c.s.f., gently dried on filter paper, weighed in small tin-foil cups on a torsion balance and then at once ground in a mortar with a small volume of N/3-HCl, boiled for 1–2 min, neutralized with N/3-NaOH after cooling, filtered and assayed for histamine. The following parts of the brain were taken:

Hypothalamus. First the hypophysis was cut off and discarded, then a nearly square tissue sample of about 2 mm depth was cut out of the hypothalamus. Its position and shape are shown in the inset of Fig. 6. The upper side of the square runs through the lower part of the massa intermedia; the lower side is formed by the floor of the third ventricle; anteriorly the square extends to a line passing through the optic chiasma and the anterior commissure, and posteriorly to a line just in front of the mamillary bodies and the beginning of the aqueduct. This tissue sample will be referred to as superficial hypothalamus. From the same site a further, deeper sample of tissue was cut out, also about 2 mm in thickness. This sample will be referred to as deep hypothalamus. In three experiments 125–150 sagittal sections of 40 μ thickness were cut out from a frozen block of the same region of the hypothalamus shown in the inset of Fig. 6. The first 40–52 sections were extracted and assayed in groups of four, the later ones in groups of eight.

Caudate nucleus. From the body of the caudate nucleus in the floor of the lateral ventricle, near the interventricular foramen, two tissue samples, a superficial and a deep, each about 2 mm thick, were removed from the same site. They will be referred to as caudate nucleus, superficial and deep.

Septum pellucidum. The medial wall of the lateral ventricle anterior to the interventricular foramen was cut out.

Massa intermedia. The massa was transected when the brain was divided in half. By cutting along the margin of the massa intermedia of the left half, a layer of tissue, about 2 mm thick, was removed.

Lateral wall of lateral ventricle. A square block of about 7 mm × 2 mm was cut from the central part of the ventricle; it contained white matter of the corona radiata.

Fimbria. The medial wall of the lateral ventricle was folded back and the exposed white tissue along the hippocampus was cut off.

Whole brain. After removal of the cerebellum and brain stem by cutting the mid-brain at the intercollicular level, the brain was divided in the mid line and each half was ground with sand in N/3-HCl about 1 ml./g. The further procedure of extraction was the same as for the tissue blocks.

Assay of histamine. The extracts of brain tissue as well as the effluent collected during and after perfusion of the cerebral ventricles were assayed for histamine on the guinea-pig ileum preparation suspended in 15 ml. oxygenated Mg-free Tyrode solution at 34° C containing atropine $1/3 \times 10^{-7}$. When extracts of brain tissue were assayed no precautions were taken to avoid interference by other substances in the extracts which either cause contraction or sensitize the preparation to histamine. When extracts yielding high histamine values per gram tissue were assayed, only small amounts of tissue (1 mg or less) needed to be added to the bath; the values so obtained can be taken as representing the true histamine content, since these amounts of tissue from a brain not perfused with histamine did not stimulate the guinea-pig gut and did not potentiate the action of histamine. On the other hand, when the tissue contained less than 5 μg histamine per gram larger amounts of extract were required for testing; the actual histamine content of the extract may therefore have been somewhat lower than the values recorded. In some of these assays giving low histamine values it was in fact found that a residual contraction remained when the extract was tested after the addition of mepyramine (0·003 μg/ml.), which abolished equivalent histamine contractions. However, since we were not interested in the exact histamine content of samples which yielded only 5 μg/g tissue or less, purification of the extracts was not attempted. All values given for histamine in this paper refer to the base.

RESULTS

Acid gastric secretion

Perfusion with histamine 1:1000 from the lateral ventricle to the aqueduct resulted in acid gastric secretion. A central effect of histamine, mediated either via the vagi or by release of adrenocorticotrophic hormone, which in its turn would release adrenocortical hormones capable of producing acid gastric secretion (French, Longmire, Porter & Movius, 1953; Porter, Movius & French, 1953), could be excluded, since the secretion occurred also after section of the vagi and after removal of the suprarenals.

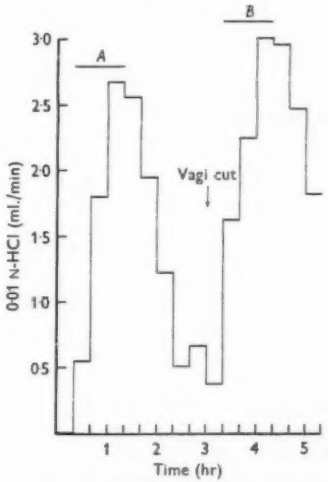

Fig. 1. Acid gastric secretion (ordinate) produced in a 2·6 kg cat under pentobarbitone sodium anaesthesia by two 1 hr periods of perfusion of the cerebral ventricles with histamine 1:1000 at a rate of 0·1 ml./min. The solid lines on top give the periods of histamine perfusion (*A*) before and (*B*) after cutting the vagi in the neck (at arrow).

Figure 1 illustrates the acid gastric secretion produced by perfusion of the cerebral ventricles with histamine, and the fact that the secretory response persists after dividing the vagi. A similar result was obtained by Bhawe (1958) with intraventricular injections of histamine.

The maximal acid gastric secretion, produced during the perfusion with histamine 1:1000, amounted in some cats to less than 0·5 ml., in others to more than 5 ml. N/100-HCl per minute. These great variations result, not from differences in the rate of absorption of histamine into the blood stream, but from differences in the sensitivity of the oxyntic cells. The acid gastric secretion, whether it was weak or strong, corresponded always to

that produced by an intravenous infusion of 1·2 μg histamine per minute. This is illustrated in Fig. 2, which gives the results obtained in three cats. In cat *A* the secretory response to perfusion of the cerebral ventricles with histamine 1:1000 for 1 hr was weak, in cat *B* it was intermediate, and in cat *C* it was strong. But the response was always of the same order as that obtained with intravenous infusion of histamine at 1·2 μg/min for 1 hr. The figure shows also that when histamine was applied by the same route for a second time the response was greater. This was usually observed.

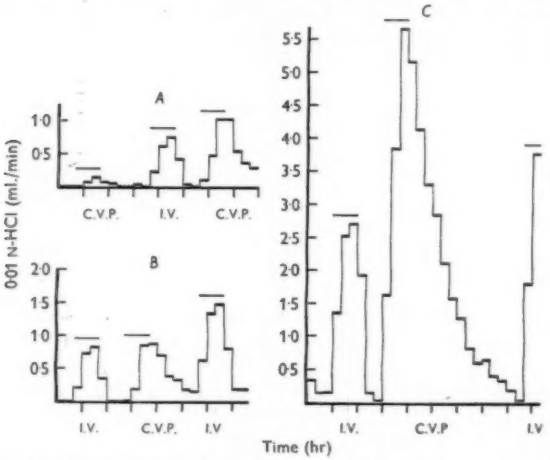

Fig. 2. Acid gastric secretion (ordinate) produced in three cats under pentobarbitone sodium anaesthesia. Comparison of the secretion produced by perfusion of the cerebral ventricles with histamine 1:1000 at a rate of 0·1 ml./min with that produced by intravenous infusion of 1·2 μg histamine per minute. (*A*) 2·6 kg cat showing a weak, (*B*) 2·6 kg cat showing an intermediate, and (*C*) 2·5 kg cat showing a strong secretory response. The solid horizontal lines give the periods of cerebral ventricular perfusion (C.V.P.) and of intravenous infusion (I.V.) of histamine.

The onset of secretion and its rise during the 1 hr periods were similar whether the hisatmine was infused intravenously or perfused through the cerebral ventricles. This was particularly striking in those experiments in which the secretory responses were small and delayed. If acid secretion failed to appear during the first 20 min period, it did not occur after histamine was administered by either route. However, since the periods of sampling of fluid from the stomach were always 20 min, differences of a few minutes in the onset of secretion would scarcely have been revealed.

There was one difference in the time course of secretion. When the intravenous infusion of histamine was stopped, the secretion decreased relatively quickly and often came to an end within 1 hr. On the other hand, on perfusion of the cerebral ventricles the maximal acid secretion was

sometimes obtained in the 20 min sample collected after the end of the histamine perfusion (see cat *B*, Fig. 2); in some experiments this sample contained as much acid as the sample collected during the last 20 min of histamine perfusion (see cat *A*, Fig. 2). Further, the acid gastric secretion continued at a slowly diminishing rate but on a low level for several hours (see cat *C*, Fig. 2).

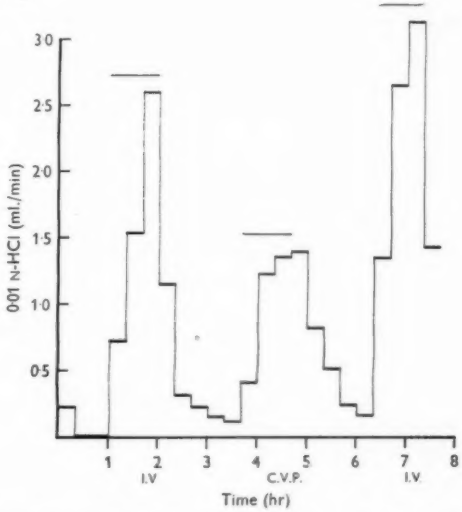

Fig. 3. Acid gastric secretion (ordinate) produced in a 2·4 kg cat under pentobarbitone sodium anaesthesia. Comparison of the secretion produced by perfusion of the cerebral ventricles with histamine 1:10,000 at a rate of 0·1 ml./min with that produced by intravenous infusion of 1·2 μg histamine per minute. The solid horizontal lines give the periods of cerebral ventricular perfusion (C.V.P.) and of intravenous infusion (I.V.) of histamine.

From the fact that the perfusion of the cerebral ventricles with histamine 1:1000 produced an acid gastric secretion similar to that obtained on intravenous infusion of 1·2 μg/min, we may conclude that during the 1 hr perfusion with 6000 μg histamine about 72 μg passed into the circulation, and even when we take into account the small additional amounts of histamine which must have entered the circulation during the following hours whilst acid gastric secretion continued at a low level, the total amount which entered the circulation cannot have been more than 100 μg histamine and was probably less.

Acid gastric secretion occurred also when the cerebral ventricles were perfused with histamine 1:10,000 but the effect was less than that produced by intravenous histamine 1·2 μg/min, as is shown in Fig. 3. Thus less histamine is absorbed than during perfusion with histamine 1:1000.

The amount absorbed, as shown by the three experiments of Fig. 4, appeared to be about 0·15 μg/min. In each experiment the maximal rate of acid gastric secretion was about 0·5 ml. N/100-HCl per minute, and this secretion was less than that produced by intravenous histamine 0·6 and 0·3 μg/min and more than that produced by 0·075 μg/min; it was about

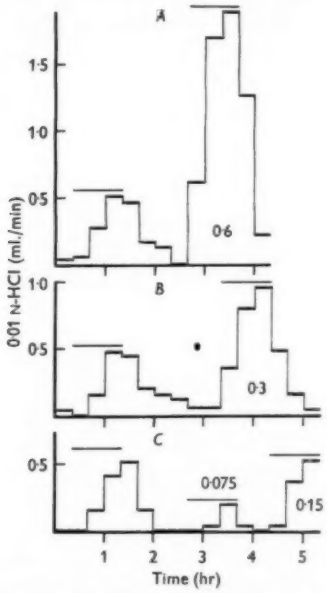

Fig. 4. Acid gastric secretion (ordinate) produced in three cats under pentobarbitone sodium anaesthesia. Comparison of secretion produced by 1 hr perfusion of the cerebral ventricles with histamine 1:10,000 at a rate of 0·1 ml./min with that of intravenous infusion. The amounts infused per minute intravenously are given by the figures in the histograms. Cat *A* (2·3 kg) 0·6 μg/min; cat *B* (2·4 kg) 0·3 μg/min; cat *C* (2·1 kg) 0·075 and 0·15 μg/min. The unmarked first response in all three cats is due to the perfusion of the ventricles with the histamine. The horizontal lines give the periods of ventricular perfusion or intravenous infusion of histamine.

equal to, or slightly greater than, that produced by 0·15 μg/min. This would correspond to a passage into the blood stream of about 10 μg histamine during the one hour from a total of 600 μg perfused through the cerebral ventricles.

Histamine is also absorbed from the subarachnoid space, since it produced acid gastric secretion when infused into the cisterna under conditions in which its entrance into the cerebral ventricles was prevented. This is shown in the experiment Fig. 5.

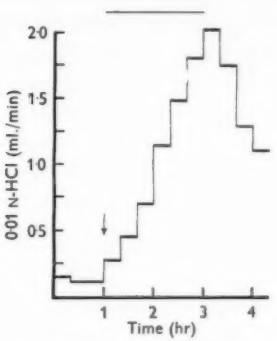

Fig. 5. Acid gastric secretion (ordinate) produced in a 2·5 kg cat under pentobarbitone sodium anaesthesia by introduction of 500 μg histamine into the cisterna. At the arrow 0·5 ml. histamine 1:1000 infused intracisternally at a rate of 0·1 ml./min. Cisternal cannula closed for 2 hr, as indicated by the horizontal line.

Recovery of histamine in aqueductal effluent

During the perfusion with histamine 1:1000 or 1:10,000 at a rate of 0·1 ml./min for 1 hr, the amount of fluid collected from the aqueduct was 6 ml. or a little more. There was therefore no net fluid loss. Nor was there any fluid loss when perfusion was continued without histamine. On the other hand, histamine could not be fully recovered from the effluent. This is shown in Tables 1 and 2.

Table 1 summarizes the results of seven experiments in which histamine 1:1000 was perfused, and gives for each experiment the histamine recovered in successive 20 min samples of aqueductal effluent. The first three 20 min samples collected whilst the 6 ml. histamine 1:1000 was perfused through the ventricles contained large amounts of histamine but, with the exception of one sample, less than 2000 μg. When perfusion was continued without histamine there was a rapid fall of histamine in the effluent. The first 20 min sample still contained between 100 and 200 μg, and in one experiment even more, but the second and particularly the third sample following the histamine perfusion usually contained less than 10 μg. Small amounts of histamine continued to appear in the effluent for several hours, as is shown in the last three experiments in the Table. By adding up these amounts it was found that the prolonged perfusion had added only another 25–45 μg to the recovery. Thus when determining the recovery in the effluent the error introduced by stopping the perfusion 1 hr after the histamine perfusion is negligible.

The recovery in the seven experiments of the Table varied between 4785 and 5761 μg (mean 5167 μg). This is a mean loss of 833 μg or of 14 %. When

considering this loss one should bear in mind the 5 % error involved in the assay of histamine. For the later samples, which contain relatively small amounts of histamine, this error would amount to a few μg only, but for the first three samples collected during the histamine perfusion, in which most of the histamine is recovered, this error could add up to nearly 300 μg.

The recovery of histamine in the aqueductal effluent during perfusion with histamine 1:10,000 is shown for four experiments in Table 2. The duration of perfusion with histamine was 1 hr in three experiments and

TABLE 1. Histamine recovered (μg) in successive 20 min samples of aqueductal effluent during perfusion for 1 hr with histamine 1:1000; total amount perfused 6000 μg

	Expt. no.						
Sample	1	2	3	4	5	6	7
1	1820	1740	1270	1670	1680	1450	1450
2	2000	1800	1670	1700	1680	1600	1670
3	1820	1600	1700	1670	1520	1660	1600
4	110	150	130	104	164	168	366
5	7	7	10	9	12	19	20
6	4	3	5	6·4	10	16	8
7	—	—	—	—	10	9	7
8	—	—	—	—	7	9·6	5
9	—	—	—	—	5	5·3	3
10	—	—	—	—	2·5	4·8	2·4
11	—	—	—	—	2·5	3·2	1·7
12	—	—	—	—	—	2·5	1·5
13	—	—	—	—	—	2·4	1·2
14	—	—	—	—	—	1·8	0·9
15	—	—	—	—	—	1·6	0·7
16	—	—	—	—	—	1·6	0·5
17	—	—	—	—	—	0·27	0·3
18	—	—	—	—	—	0·21	0·2
19	—	—	—	—	—	0·16	0·12
20	—	—	—	—	—	—	0·075
Total recovery	5761	5300	4785	5159	5094	4895	5139

TABLE 2. Histamine recovery during perfusion with histamine 1:10,000; histamine content (μg) in successive 20 min samples of aqueductal effluent. In Expts. 1–3, perfusion with histamine was continued for 1 hr, in Expt. 4 for 2 hr. Total amount of histamine perfused, 600 and 1200 μg respectively

	Expt. no.			
Sample	1	2	3	4
1	145	148	145	145
2	170	167	145	160
3	175	167	150	160
4	14	14·8	17	145
5	3	1·5	3	145
6	1·4	0·64	—	160
7	0·8	0·34	—	11·6
8	0·44	0·21	—	2·2
9	0·28	0·11	—	—
10	0·14	—	—	—
Total recovery	510	500	460	929

2 hr in one. The time course of recovery was similar to that found in the perfusions with histamine 1:1000. The loss in the three experiments, in which 600 μg histamine was perfused during 1 hr, was between 90 and 140 μg, and in the one experiment in which 1200 μg was perfused during 2 hr, the loss was 271 μg. The mean loss in all four experiments was 19 %.

Protein content. When an equal amount of concentrated nitric acid was added to a sample of c.s.f. collected from the cisterna a faint but definite opalescence was obtained, indicating the presence of small amounts of protein in the c.s.f. This test was always negative when applied to samples of aqueductal effluent collected during perfusion of the cerebral ventricles,

TABLE 3. Histamine content (μg/g fresh tissue) of various parts of the left half of the brain and of the choroid plexus after perfusion for 1 hr of the cerebral ventricles with histamine 1:1000 from the cannulated left lateral ventricle

Tissue samples	Expt. no. 1	2	3	4	5	6	7	8	9	10	Mean
Hypothalamus (superficial)	104	114	111	137	111	—	160	173	333	148	155
Hypothalamus (deep)	—	50	59	44	23	—	—	—	—	—	44
Massa intermedia	—	—	—	—	—	57	142	—	55	52	77
Septum pellucidum	320	266	125	242	182	200	363	470	250	200	262
Caudate nucleus (superficial)	—	200	172	250	211	143	363	190	333	225	232
Caudate nucleus (deep)	—	—	30	50	32	—	—	—	—	—	37
Lateral wall of lateral ventricle	16	50	11	80	—	—	—	—	76	40	46
Fimbria	—	—	45	235	27	32	222	47	31	111	94
Choroid plexus	33	15	25	57	32	—	—	—	22	57	34

whether with or without histamine. A different result was obtained in the experiments in which 500 μg histamine in 0·2 ml. artificial c.s.f. was injected, either into the ventricle or into the cisterna. The fluid was kept in these spaces so as to allow any protein to accumulate. When the aqueductal or cisternal cannula was opened 2 hr later, between 0·4 and 1·2 ml. of fluid flowed out under pressure. This fluid contained large amounts of protein and gave a heavy precipitate when treated with concentrated nitric acid. Such an effect did not occur when 0·2 ml. artificial c.s.f. without histamine was injected intraventricularly, although there was also a similar rapid outflow of fluid when the aqueductal cannula was opened 2 hr later; this fluid gave a just-perceptible opalescence when treated with concentrated nitric acid. This shows that histamine leads to the appearance of large amounts of protein in the c.s.f.

Uptake of histamine by brain tissue

The histamine content of the various samples of brain tissue examined after 1 hr perfusion of the cerebral ventricles with histamine 1:1000 is shown in Table 3. The content is expressed in μg/g fresh tissue.

High histamine values (between 104 and 470 μg/g) were regularly found in the samples of tissue taken from regions of grey matter lining the ventricles; i.e. hypothalamus (superficial), caudate nucleus (superficial) and septum pellucidum. In all but one experiment the values for the caudate nucleus and septum pellucidum were higher than for the hypothalamus. But there were no great differences between caudate nucleus and septum pellucidum, sometimes the one and sometimes the other giving the higher value.

The deeper layers of the hypothalamus and caudate nucleus contained 23–59 μg histamine per gram. Although these values are lower than those of the corresponding superficial tissue layers, they provide evidence that histamine had passed from the ventricular surface into these distant layers of grey matter, since their normal histamine content was 5 μg/g or less.

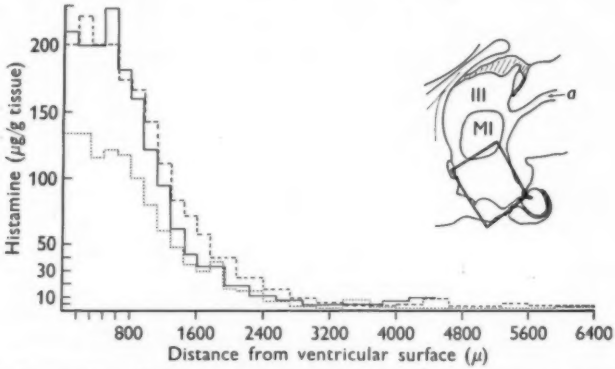

Fig. 6. Penetration of histamine into the wall of the hypothalamus after perfusion of the lateral ventricles with histamine 1:1000 for 1 hr. Three different experiments: —— Expt. *A*, – – – Expt. *B*, · · · Expt. *C*. Histamine content in μg/g of frozen sections cut parallel to the ventricular surface. Inset: diagram of the medial aspect of the diencephalon; the block for tissue sections was taken from the area indicated by the square.

In three experiments the penetration of histamine was studied in more detail in frozen sections of the hypothalamus cut parallel to its ventricular surface. The results are given as histograms in Fig. 6. In experiments *A* and *B* the histamine content of the first 16 sections was 200 μg/g or more; thereafter the histamine values fell rapidly with each group of four sections, and values of less than 5 μg/g were obtained after about 80 sections. In experiment *C* the histamine content of the first sections was only just over 130 μg/g, but in the subsequent sections it declined about as rapidly as in the other two experiments. From these results it is evident that histamine penetrates into the hypothalamus for at least 2·5 mm.

In experiment *A*, after the last frozen section was cut, the remainder of the block was fixed in formalin for histological examination and the first section cut from this block was stained for cells and fibres with Luxol Fast Blue according to the procedure of Klüver & Barrera (1953). It corresponded in Hess's atlas of the cat's brain (Hess, 1932, 1956), to the sagittal section in plane 180, which is over 4 mm from the mid line. This agrees well with the number of frozen sections cut, since 112 sections of 40 μg had been removed before the block was fixed in formalin.

In experiment *A*, after the histamine value of the frozen sections had fallen to 4·4 μg/g, it started to rise again and reached a value of 9·5 μg/g in the last 8 frozen sections. In the other two experiments there were also small transient increases in the histamine values after they had fallen to a low level. These rises cannot be fully explained. They may reflect a penetration of histamine from the lateral ventricle; for instance, in experiment *A*, in which the remainder of the block was fixed in formalin, the last section had actually passed through the tip of the inferior horn. Or they may reflect the presence in these regions of the brain of larger amounts of naturally occurring gut-stimulating substances other than histamine, such as substance P or 5-HT. The problem has not been further examined.

The massa intermedia was examined in four experiments. In three the histamine values were just over 50 μg/g, which corresponds to the values obtained for the deeper layers of the hypothalamus and caudate nucleus. In one experiment the value was 142 μg/g. This high value could be the result of a more effective penetration of histamine in this particular experiment, but it could just as well be due to the fact that the piece of tissue removed contained a larger proportion of tissue from near the ventricular surface.

The white matter of the lateral wall of the central part of the lateral ventricle yielded in each experiment considerably lower histamine values than the grey matter lining either the lateral ventricle (septum pellucidum, caudate nucleus) or the third ventricle (hypothalamus). This finding suggests that histamine passes less readily into the white matter than into the grey. The values showed great individual variations, i.e., between 11 and 80 μg/g. The lowest value, of 11 μg/g, was obtained in an experiment in which the values for other parts of tissue lining the lateral ventricle were also low in comparison with other experiments. Perfusion of the ventricle may thus have been less thorough in this experiment than usual. This explanation would not apply to the second-lowest value of 16 μg/g obtained for the white matter of the lateral wall. If the histamine were to pass only a very short distance into the white matter, small differences in thickness of the tissue samples would greatly affect their histamine value and differences in thickness of the tissue samples taken may therefore have contributed to the wide individual variations observed.

The greatest individual variations were obtained with fimbria which consists of white matter. The values varied between 27 and 237 μg/g. On

the assumption that the histamine passes less readily into white than into grey matter, low values would have been expected, and were in fact obtained in five out of eight experiments. An explanation for the two exceptionally high values may be the thinness of the fimbria.

No histamine had apparently reached the floor of the fourth ventricle, the cerebellum and the suprasylvian or lateral gyrus of the cerebral cortex, since the histamine equivalents obtained with tissue samples from these regions, which are far removed from the perfused ventricular surface, were between 1 and 6 μg/g. Values of the same order were also obtained in corresponding tissue samples obtained in control experiments in which the ventricles had been perfused with artificial c.s.f. not containing histamine. Since relatively large amounts of tissue extracts had to be used for the assay of these tissue samples their actual histamine content may have been even less than these low values (see Methods).

TABLE 4. Histamine content (μg/g fresh tissue) of various parts of the right half of the brain and of the choroid plexus after perfusion of the cerebral ventricles with histamine 1:1000 from the cannulated left lateral ventricle. The experiment numbers refer to those in Table 3

Tissue samples	Expt. no. 3	4	5	9	10	Mean
Septum pellucidum	45	73	21	30	22	38·0
Caudate nucleus (superficial)	—	—	—	17	5	11·0
Lateral wall of lateral ventricle	—	—	—	8	5	6·5
Fimbria	—	29	—	2	—	15·5
Choroid plexus	—	—	—	9	5	7·0

In a few experiments tissue samples were taken from the non-cannulated, right half of the brain. The histamine values are given in Table 4 and are lower than those of the corresponding tissue samples from the cannulated side. This is not surprising, since experiments in which a 0·2% solution of Evans Blue was perfused through the cerebral ventricles had shown that the lateral ventricle was less stained on the non-cannulated than on the cannulated side; the staining was often slight and sometimes even absent. The uneven perfusion did not extend to the third ventricle and accordingly the values for the superficial hypothalamus were also high when taken from the right half of the brain. In fact, the values for experiments 7 and 8 in Table 3 were obtained from the right side and the value for experiment 2 from both sides of the hypothalamus.

In several experiments the histamine content of the choroid plexus of the left lateral ventricle was determined; sometimes the samples included the plexus of the third ventricle. They contained between 15 and 57 μg/g (mean 34 μg/g). These values indicate an uptake of histamine from the perfusion fluid, since the histamine equivalents for choroid plexus in two experiments in which the ventricles were perfused with artificial c.s.f. not

containing histamine were 8 and 7 μg/g only. There was no appreciable uptake of histamine by the choroid plexus of the non-cannulated ventricle, for in two histamine perfusion experiments in which the choroid plexus of the right lateral ventricle was also examined its histamine values were 9 and 5 μg/g.

In three experiments the histamine content of both halves of the brain was determined without the cerebellum, the brain being cut at the intercollicular level just caudal to the tip of the aqueductal cannula. The results are shown in Table 5. The mean histamine content of both halves together was 116 μg. The actual histamine uptake by the brain is likely to be lower, since no corrections were made for the normal histamine content of the brain or for sensitizing substances present in brain extracts.

TABLE 5. Histamine content of brain after perfusion of the cerebral ventricles from the cannulated left ventricle with histamine 1:1000 for 1 hr

	Histamine in brain (μg)			Deficit of histamine in aqueductal effluent (μg)
Expt. no.	Left half	Right half	Total brain	
1	60	49	109	620
2	71	27	98	840
3	121	20	141	653

In the three experiments of Table 5 the histamine was also assayed in the aqueductal effluent. As in other experiments of this kind, the 6000 μg histamine infused during the 1 hr period was not fully recovered in the effluent and the last column gives the loss for each experiment (averaging 704 μg). Since the histamine recovered from the brain was of the order of 100 μg and the absorption into the blood stream, as measured by the acid gastric secretion, accounted for at most another 100 μg, there remains an unaccounted loss of about 500 μg histamine. A few preliminary experiments were carried out to see whether this loss could be explained by enzymic destruction of histamine. However, when histamine was added to homogenized brain tissue or tissue of the choroid plexus and incubated for 1 hr at 37° C it was recovered almost quantitatively.

DISCUSSION

The present experiments show that histamine can be absorbed into the blood stream from the cerebral ventricles without entering the subarachnoid spaces. In addition, on its passage from the lateral ventricle to the aqueduct, histamine is taken up by the brain tissue and by the choroid plexus.

Histamine could have been absorbed into the blood stream by capillaries of either the choroid plexus or of the brain tissue itself. If the absorption

took place via the choroid plexus the histamine would have to pass through a layer of epithelial cells and then through a layer of connective tissue before reaching the capillary structures. If the absorption were through the brain capillaries, the histamine would also have to pass first through an epithelial layer, the ependyma, and then through a layer of nervous tissue. Since histamine was shown to pass into the choroid plexus as well as into the brain substance surrounding the ventricles it is not possible to state whether the passage into the blood is through the vessels of the choroid plexus or of the brain tissue or of both.

Histamine can also be absorbed from the subarachnoid space. This is shown by the fact that acid gastric secretion occurs also on injection of histamine into the cisterna under conditions which prevent its entrance into the cerebral cavities. The histamine may either enter directly into the small vessels which are so abundant in the subarachnoid spaces, or it may be absorbed by special structures such as the arachnoid villi. Some absorption may also occur via those parts of the choroid plexus which protrude through the lateral recesses into the subarachnoid space. It is, however, not necessary to assume that histamine reaches the epicerebral cranial spaces and is then absorbed via the endocranial sinuses, since we found that dye injected intracisternally under similar conditions did not reach the upper surface of the cerebral hemispheres.

The uptake of histamine from the ventricles into the brain tissue lining these cavities and its deep penetration into the grey matter within one hour is of interest in connexion with the fact that substances injected into the ventricular system of conscious cats produce profound changes in behaviour. These changes resemble in many respects those which Hess (1932, 1956) obtained in his classical experiments on electrical stimulation of diencephalic structures. In Hess's experiments the stimulating electrodes were placed at different points of the diencephalon, but responses were obtained predominantly from points within 3 mm of the mid line, i.e., from a region which can be easily reached by substances penetrating from the cerebral ventricles, as is shown by the present experiments with histamine.

The finding that the uptake of histamine by brain tissue occurs more readily in grey than in white matter, and the observation that there are also differences in histamine uptake between different regions of grey matter lining the ventricles, are at variance with conclusions reached by other authors. For instance, Spatz (1933) concludes that the penetration of dyes from the outside or inside of the brain surface progresses on a broad front independent of any structural differences. The brain behaves, as he says, like a colloidal mass. More recently Bakay (1956) states that 'we have no reason to believe that an isotope follows any particular path-

way of absorption within the brain. The process starts with a diffuse inhibition of the superficial layers. In microscopic radio-autographs P^{32} seems to invade the brain from the cerebrospinal fluid diffusely.'

A different result, however, was recently obtained by Roth, Schoolar & Barlow (1959), who injected labelled acetazoleamide (Diamox) intravenously into cats and found that it is first secreted into the cerebral ventricles and then passes into the brain tissue. This uptake was found to be more pronounced in the grey matter lining the ventricles and the aqueduct than in the white matter. Similarly when the Na-salt of the dye bromophenol blue is perfused through the cerebral ventricles, it is also taken up predominantly by grey matter (Feldberg & Fleischhauer, 1959). The fact that not only histamine but other substances as well pass more readily from the ventricular cavities into the grey matter than into the white suggests, therefore, that it is not the ability of histamine to increase capillary permeability which is the cause of its deep penetration into the grey matter. Nevertheless, histamine increases capillary permeability when injected into the cerebral cavities or into the subarachnoid space. This is evident from the appearance of protein in the ventricular and subarachnoid fluid after such injections. Histamine may also increase the permeability of ependymal cells, which might facilitate its passage into the brain tissue, but this could not explain the differences in penetration of grey and white matter.

SUMMARY

1. In cats anaesthetized with pentobarbitone sodium the cerebral ventricles were perfused from a cannula implanted into the left lateral ventricle to the cannulated aqueduct with solutions of histamine 1:1000 or 1:10,000 at a rate of 0·1 ml./min and for periods of 1 hr.

2. The histamine perfusion caused acid gastric secretion. This secretion was not a central effect of histamine mediated either via the vagi or by a liberation of adrenocorticotrophic hormone and subsequent release of adrenal cortical hormones. The secretion is attributed to absorption of histamine from the ventricles into the blood stream.

3. The amounts of histamine absorbed from the ventricles into the blood stream were assessed by comparing the secretion with that produced by intravenous infusions of histamine. On perfusion of the ventricles with histamine 1:1000 the absorption corresponded to about 1·2 μg/min, or during perfusion for 1 hr to less than 100 μg. On perfusion with histamine 1:10,000 the absorption corresponded to about 0·15 μg/min, or during perfusion for 1 hr to about 10 μg.

4. Histamine was also absorbed from the subarachnoid space, as is shown by the fact that a cisternal injection of 500 μg histamine, under

conditions which prevented its entrance into the cerebral ventricles, produced acid gastric secretion.

5. After perfusion of the cerebral ventricles for 1 hr with histamine 1:1000 histamine was found to have penetrated into the brain tissue from the ventricular surfaces. More histamine was taken up by grey than by white matter.

6. The penetration of histamine into grey matter was determined in greater detail by assaying the histamine content of frozen sections of the wall of the hypothalamus cut parallel to the ventricular surface. Histamine penetrated to a distance of at least 2·5 mm from the surface.

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THE AFTER-EFFECTS OF A WHITE LIGHT STIMULUS

By PATIENCE W. TREZONA
From the Physiological Laboratory, University of Cambridge

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The subject of after-effects following a bright light stimulus is notable for the profusion of its literature, for the detailed accounts of the phenomena observed and for the contradictory nature of these accounts. The observations have mostly been qualitative and have seldom specified the exact conditions of observation. In a large proportion of them the 'after-image' is viewed on a light background—i.e. a second stimulus of larger field is introduced. This more complicated state of affairs is not considered in the present work.

Of the investigators who have viewed the after-effects in darkness many find an intermittent after-effect. Duke-Elder (1932) gives the following after-effects, basing them on the work of Fröhlich and Judd: (1) a primary image followed by a short dark interval; (2) a first positive (Hering) after-image: (3) a second dark interval; (4) a second positive (Purkinje) after-image; (5) a third long dark interval; (6) a third positive after-image and finally (7) a long dark (negative) phase. On the other hand, Padgham (1953) finds a continuous effect on the removal of the stimulus. He records no dark intervals but only slight decreases in intensity, the effect lasting for about a minute.

The present investigation was undertaken in order to try to reconcile such conflicting reports as the above and to see what different effects occur under different experimental conditions. The field size, the retinal illumination, the time of exposure and the part of the retina stimulated can all be varied. Only white lights have been used in the results here discussed. The investigation was conducted in such a way as to be independent of colour naming as far as possible. The main technique was to judge whether or not each effect was present.

METHODS

Apparatus

The apparatus is shown in Fig. 1 and utilizes the Maxwellian view. A 6 V, 108 W ribbon-filament lamp S_1 (illuminant S_A) is focused by a condenser lens L_1 on to the exit pupil E_1 of diameter 2 mm, which is completely filled by the image of the ribbon. Close to the lens L_1 is an electrically operated shutter X_1, an aperture A and filters F. A spectacle lens L_2 brings the aperture A into

focus by the unaccommodated eye E. A red point source S_2 obtained from a torch bulb and red filter is reflected by a cover-slip at C, the image being in the plane of A. The observer's head is fixed by an adjustable dental impression and the right eye is always used.

Shutter. An electrically operated shutter X_1 is used and can be set into operation by the observer. The following 'speeds' can be obtained: $\frac{1}{2}$, 1, 2, 4, 8, 16, 32, 64 sec. The shutter can be hand operated for longer times if necessary.

Filters. The neutral density filter combinations normally used give nominal densities of 0·6, 1·2, 1·8, 2·4...9·0, 9·6 log. units.

Apertures and fixation spot. For most of the investigation apertures at A consist of a single circular hole subtending the following field sizes at E_1—$\frac{1}{2}°$, 1°, 2°, 3°, 4°, 5°. The lamp S_2 with a red filter is adjusted in position so that the red fixation spot appears at the centre of the field; its brightness is always adjusted to threshold value. For measuring the threshold of vision at the fovea for small fields the single fixation spot is replaced by four; these form a square and the field is located at the centre.

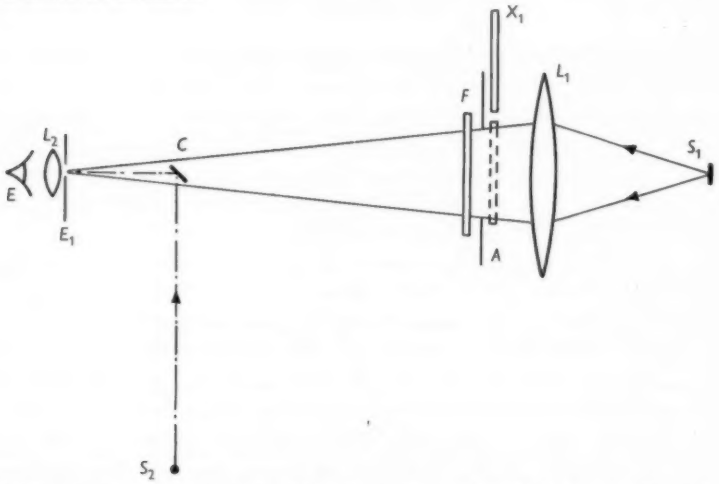

Fig. 1. Diagram of the apparatus. S_1, ribbon-filament lamp; S_2, red point source; L_1, condenser lens; L_2, spectacle lens; X_1, electrically operated shutter; A, aperture; F, filters; C, cover-slip; E_1, artificial pupil; E, eye.

For extra-foveal work on $\frac{1}{2}°$ and 1° fields this arrangement is changed, the fixation spot as well as the field being inserted at A. Two sets of plates were made, one for the $\frac{1}{2}°$ and the other for the 1° field, with fixation spots at 1°, 2°, 3° 4° and 5° off the field and such that the field falls on the nasal side. The fixation spot again has a red filter and can be adjusted in brightness independently of the field.

Calibrations

The retinal illumination produced was found from the flux coming through the exit pupil together with the appropriate distance measurements. The flux was measured with a corrected photocell and microammeter, previously calibrated. These were also used to measure the densities of the neutral filters.

The observers

Owing to the long time involved in the investigations it was not possible to perform each one on a large number of subjects. P.W.T. made all the observations and each set was repeated on B.L. or D.M.W. In addition, further subjects verified the salient points; these were the above

and E.N.W., D.M.L., M.R.H.W. and T.W. All were normal except P.W.T., who is slightly protanomalous. In the type of observation made this defect would not be expected to be of great importance and the good agreement reinforced this view. All observers were given one or two practice sessions.

The observers' tasks. Each session lasted 1–1½ hr, of which 10 min was used for dark adaptation: for all observations near the threshold of vision there were 30 min dark adaptation. The different types of field presented for the observers' consideration were given at random.

Although the observer was guided into giving the type of decision required by the investigation, care was taken not to suggest to him what he was likely to see. For this reason the enquiry went similarly to the following example. The subject was first asked what he saw after the light went out. He might reply that he saw a blue circle. Only then was he asked to time the duration of the blue circle, although the investigator knew from the beginning that the duration of the blue circle was the information that he required. Since the readings needed a considerable amount of concentration, the subject was not asked for too much information at one time; he was often given a separate viewing to make a judgement on the presence of a second effect from that in which he judged the first.

Definitions

After some experience in viewing after-effects it was found that, broadly speaking, each effect fitted into one of several categories. Sometimes there are 'light' after-effects and sometimes 'dark' after-effects. In each of these the size and shape of the field itself is recognizable in the after-effect, but in the former the after-effect is lighter than the surroundings while in the latter it is darker.

Where these after-effects are lighter than the surroundings, the following different types can be seen.

Persistence of vision. When the light is extinguished it appears to continue in the same colour, but with rapidly decreasing intensity, for a second or less. This phenomenon lies in quite a different category from the other after-effects and is only considered incidentally in this paper.

After-colours. When a bright white light is extinguished, one can see for many seconds or even minutes a coloured 'after-image'. There are many changes of colour, usually with no gap between one colour and the next and with no latency of onset.

After-blueness. This lasts for only a few seconds and remains the same colour—usually blue but sometimes grey or purple. It may be *continuous* or *intermittent.* Continuous after-blueness stays at the same intensity throughout, usually with no latency of onset. Intermittent after-blueness comes and goes, usually on for about a second and then off for about a second, for 1, 2, 3 or more cycles.

When after-effects are darker than the surroundings they are of one type only.

After-blackness. In this condition only blackness can be experienced but the area previously occupied by the stimulus is blacker than the rest. There may be 1, 2 or more of these black 'after-images' in succession and they may occur alone or following a 'light' after-effect. They must not be confused with 'negative after-images' which occur in a previously stimulated region when a second, larger stimulus is viewed.

TABLE 1

After-effects

'Light' after-effects	(Persistence of vision)
	After-colours
	After-blueness { Continuous / Intermittent
'Dark' after-effects	After-blackness

The above effects are summarized in Table 1. These are the main after-effects observed after viewing a white light stimulus under different conditions; some conditions elicit a certain after effect in a much more clear and typical form than others. In addition there are other less

prominent or rare after-effects which no attempt has been made to classify or study. Also, there are sometimes spurious after-effects—those that are neither reproducible nor related to the size and shape of the field of view and which often move across the field.

RESULTS

Field size and retinal illumination varied

In this experiment all fields were viewed foveally and the exposure time was 8 sec. Results are shown in Fig. 2, where the points represent for various field sizes the threshold retinal illumination below which after-effects of various types were not observed.

It can be seen that the threshold of after-colours stays remarkably constant for different field sizes and there is good agreement between the two observers. Every time that after-colours was seen, the stimulus had produced discomfort; this was not the case with after-blueness. Padgham (1953), who described after-colours, worked with retinal illuminations well above this threshold.

Below this critical level it is interesting to find that for field sizes of $\frac{1}{2}°$ and 1° there is no 'light' after-effect except sometimes persistence of vision; but for the 2° field and up to 5°, the limit of the present experiments, after-blueness is observed over a considerable range of retinal illumination. It is not, however, of the same type throughout the range. At low values of retinal illumination the after-blueness is of the continuous type, but for higher values it becomes intermittent. As the retinal illumination increases the number of intermittent images also increases. The number for a given value of retinal illumination is not very repeatable but there are about three at 4·5 log. trolands but only one at 2·6 log. trolands.

The threshold of after-blueness is fairly constant for field sizes of 2°–5°; it is perhaps a little lower for the larger fields but no more than would be expected if an area-summation effect were in operation. Below this threshold there is no 'light' after-effect or persistence of vision only.

The results of this experiment were verified by four other observers—E.N.W., D.M.L., M.R.H.W. and T.W. Each subject was asked to comment on four combinations of retinal illumination and field size, one from each of the regions *A*, *B*, *C*, *D* (Fig. 2*c*). Each observer gave 'after-colours', 'after-colours', 'no light after-effects' and 'after-blueness', respectively.

Figure 2 also shows that 'light' after-effects start at 3–4 log. units above the threshold of vision.

Retinal position and retinal illumination varied

The results of Fig. 2 show that after-blueness is obtained foveally, only for 2° fields and larger. This gives rise to the question of whether it is the larger field or the more peripheral component which causes them. The next experiment, therefore, kept the field size constant and varied the retinal position and

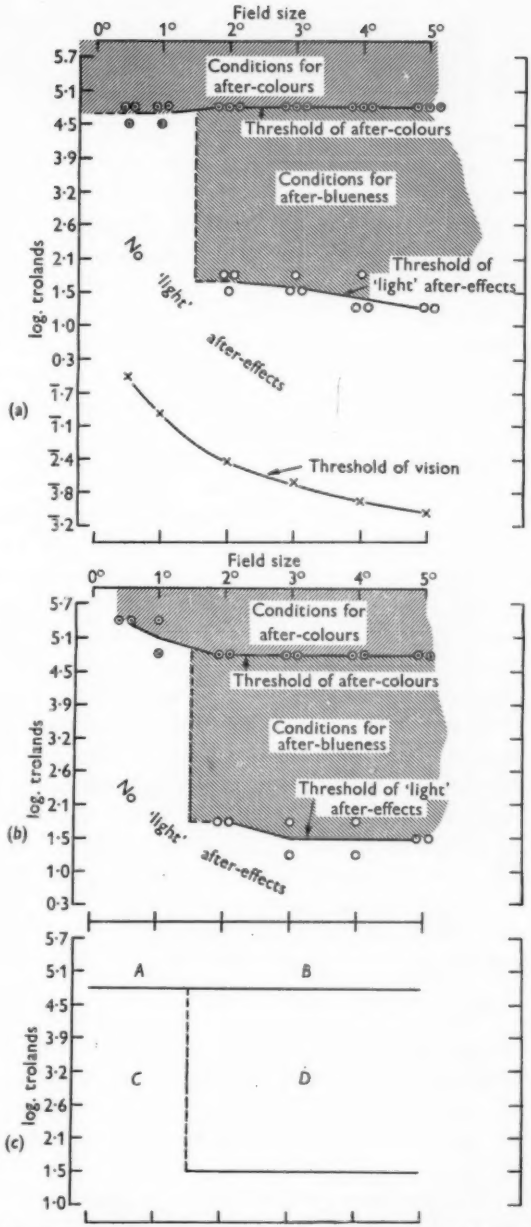

Fig. 2. Thresholds of 'light' after-effects for fields of varying size and log. trolands, viewed foveally and exposed for 8 sec. A point is shown between two given values of log. trolands when the effect was visible at the higher but not at the lower level; it is shown on the given value when here its existence was in doubt. The points are displaced slightly for clarity. Thresholds are of after-colours ⊙, after-blueness ○, and the threshold of vision ×. (*a*) P.W.T.; (*b*) B.L.; (*c*) Diagrammatic summary (see text).

retinal illumination. The fields subtended 1° and ½° and the retinal image fell foveally and at varying positions up to 5° on the nasal side.

Results are shown in Fig. 3. The abscissa represents the position off the fovea but otherwise points are plotted as described for Fig. 2. The threshold 'light' after-effect is after-blueness in the periphery and after-colours near the fovea, where the curve shows a sharp rise. This rise suggests that it is retinal position rather than field size which causes the abrupt change in Fig. 2.

The threshold of after-blueness stays fairly constant. It was too difficult to find the peripheral change-over between after-blueness and after-colours because of the presence of some scattered light which is particularly troublesome under these conditions. Scattered light sometimes gives large-field after-effects which complicate the issue.

The observer B.L. seemed more critical than P.W.T. B.L.'s after-blueness persisted to within 1° of the fovea, being absent only for the foveal observation; but P.W.T. with a 1° field showed after-blueness at 3° off the fovea, was doubtful at 2° off and did not show it at 1° off. Also, while B.L. responded similarly to the ½° and 1° fields, P.W.T. found the ½° field much more difficult than the 1° field. Sometimes she saw peripheral after-blueness and sometimes not. Thus ½° must be her change-over size between having after-blueness and not having it; for B.L. this critical field size must be smaller.

The abrupt change in threshold of the 'light' after-effects with retinal position for the 1° field was verified by E.N.W., D.M.L. and M.R.H.W. by presenting them with a few suitably chosen fields. But for the ½° field, out of a total of five observers, two saw after-blueness clearly, two doubtfully and one not at all. It appears, then, that for all observers, given a suitable retinal illumination and an adequate field size, the presence of after-blueness depends on retinal position; for some observers the ½° field is adequate, while for others it is not.

After-blackness which follows 'light' after-effects has a slightly lower threshold. For the 1° field its threshold is the same foveally as peripherally.

Exposure time and retinal illumination varied

Figure 4 shows the effect of varying the exposure time and retinal illumination of a 5° field viewed foveally. Figure 4 (*a*) and (*b*) show two separate experiments by D.M.W. and (*d*) and (*e*) show two by P.W.T. Besides considering the presence of after-blueness and after-blackness, the duration of the former was timed (Fig. 4 (*c*), (*f*)).

For low retinal illuminations, continuous after-blueness and after-blackness occur separately, the former for short exposure times only and the latter for long exposures only; for intermediate exposure times there are no after-effects. For medium retinal illuminations, continuous after-blueness has extended to

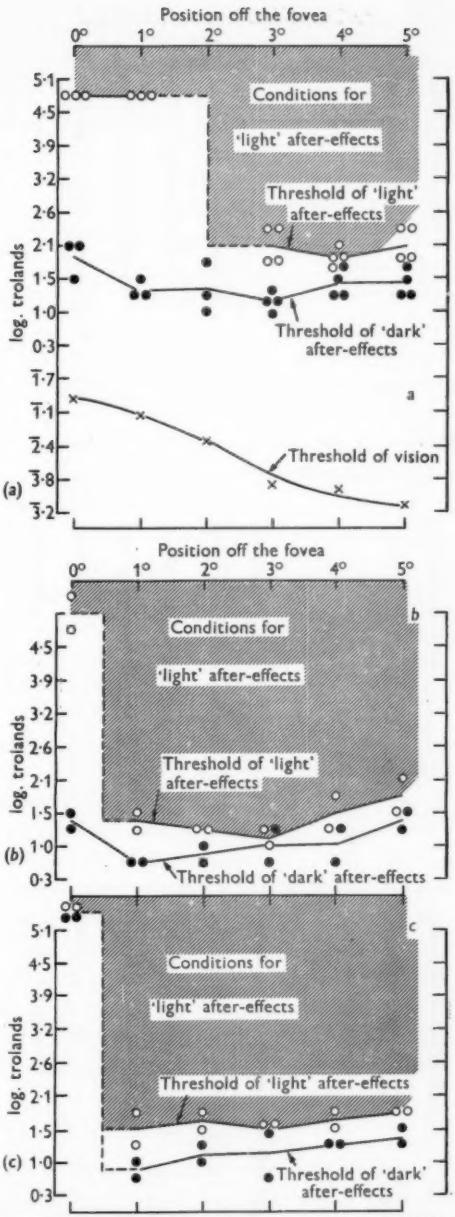

Fig. 3. Thresholds of 'light' after-effects (○) and 'dark' after-effects (●) for small fields, exposed for 8 sec, at various positions off the fovea and for different log. trolands. (*a*) P.W.T. 1° field; (*b*) B.L. 1° field; (*c*) B.L. $\frac{1}{4}$° field. Threshold of vision × given for P.W.T. Points plotted as for Fig. 2.

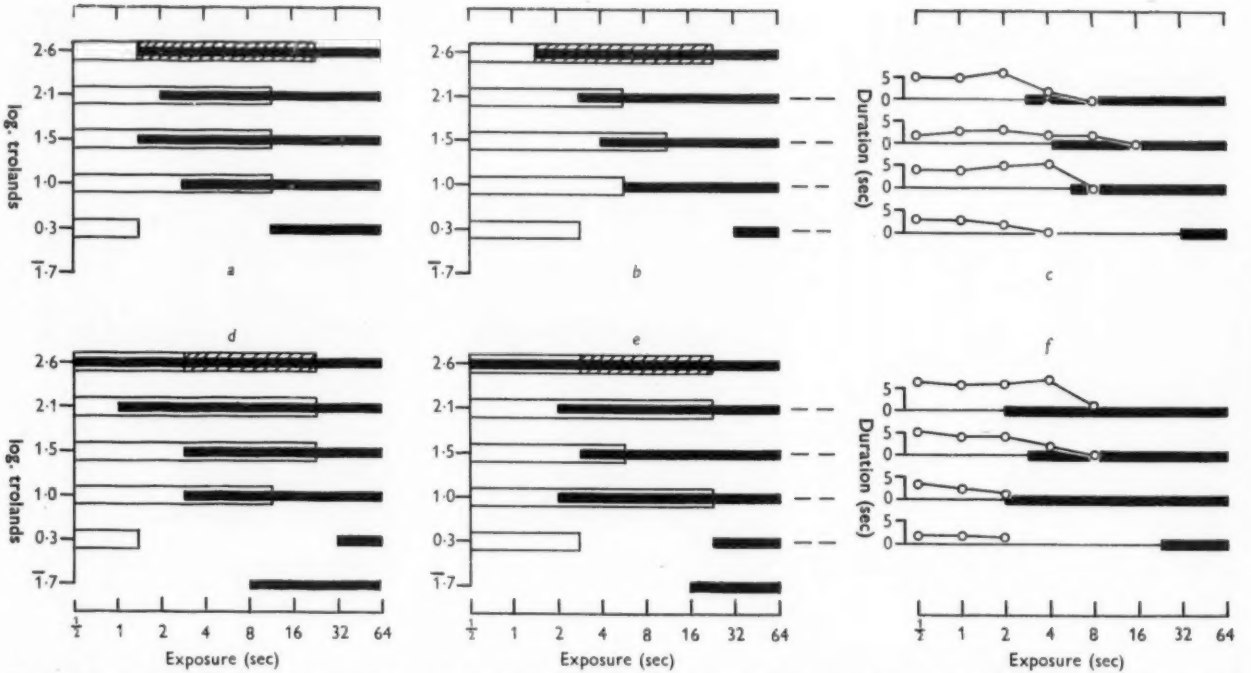

Fig. 4. Occurrence of after-blueness (rectangle) and after-blackness (thick line) for a 5° field viewed foveally for different log. trolands. (*a*) and (*b*) D.M.W.; (*d*) and (*e*) P.W.T. After-blueness may be continuous (clear rectangle) or intermittent (hatched rectangle). An effect is plotted between two given exposure times when it was visible at one and invisible at the other and on the given exposure when here its existence was in doubt. The duration of after-blueness by D.M.W. (*c*) and P.W.T. (*f*) is also shown. The timings of (*c*) were part of the investigation of (*b*) and those of (*f*) were part of (*e*).

longer exposure times and after-blackness has extended to shorter; for intermediate exposure times continuous after-blueness occurs, followed by after-blackness. For higher retinal illuminations and intermediate exposure times intermittent after-blueness occurs.

The timing of continuous after-blueness (Fig. 4 (*c*), (*f*)) showed that in many cases while its duration stays fairly constant for short exposures, it rapidly becomes shorter when after-blackness also is present. This suggests that after-blackness inhibits after-blueness. If this occurs for 2·1 log. trolands, inhibition is likely to be present also at 2·6 log. trolands and may be partly responsible for the intermittence of after-blueness here. Thus intermittent after-blueness would be derived from two processes, continuous after-blueness and after-blackness, the after-blackness inhibiting the after-blueness and the latter reappearing afterwards.

An alternative explanation of the intermittence has been made, that binocular rivalry is responsible, the after-effect of the field of the right eye alternating with the darkness of the left eye. To test this idea, the field was presented to both eyes but the intermittence remained. Thus it would appear that binocular rivalry is not the cause.

The following effects were verified by E.N.W., D.M.L. and M.R.H.W. The actual exposure time and retinal illumination were varied slightly to make the effect most definite to the individual observer:

(1) for medium retinal illumination and short exposure time—continuous after-blueness;

(2) for medium retinal illumination and long exposure time—after-blackness;

(3) for medium retinal illumination and medium exposure time—continuous after-blueness followed by after-blackness;

(4) for higher retinal illumination and medium exposure time—intermittent after-blueness followed by after-blackness.

A few observations were also made by B.L. and P.W.T. on a 1° field at 4° off the fovea when the exposure time and retinal illumination were varied. Figures 2 and 3 have shown how a small field off the fovea behaves in the same way as a large field viewed foveally, in that they both give after-blueness. Now this further experiment showed that they are also similar in giving, at a suitable retinal illumination, continuous after-blueness for short exposure times, after-blackness for long exposures and both for intermediate times.

DISCUSSION

The results of this work lie in two main groups. The first concerns the changes which occur when the retinal illumination is varied. The after-effects remain 'light' but change from after-blueness to after-colours; this general picture is modified by changes of field size or field position. The second concerns changes

which occur when the stimulus exposure time is altered. The after-effects change from 'light' to 'dark' ones; a change of retinal illumination modifies this general picture.

In the first group after-colours occur for high retinal illuminations for all field sizes and all retinal positions; after-blueness occurs for lower retinal illuminations, only if the field is viewed peripherally or if a foveally viewed field is 2° or larger. Thus a peripheral component has to be present for after-blueness to occur. This fact, together with the blue or grey colour and occurrence at lower retinal illuminations, suggests that rods are responsible for after-blueness. Similarly, with after-colours the varying colours, the foveal as well

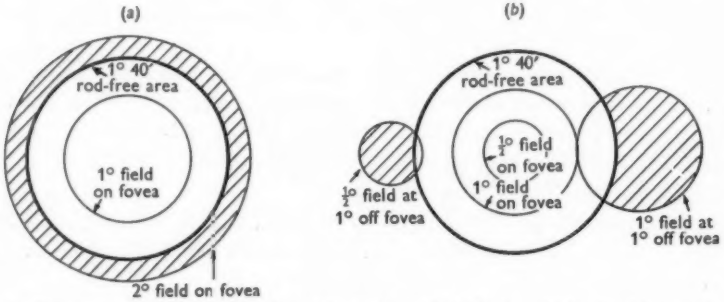

Fig. 5. Diagram to compare Polyak's rod-free area with fields for which after-blueness is just seen and just not seen. (*a*) Foveally (see Fig. 2*a*, *b*). (*b*) Off the fovea (see Fig. 3*b*, *c*). Shaded areas show where rods can be expected in fields giving after-blueness.

as peripheral occurrence and the high retinal illumination required suggest that cones are responsible for this. However, this work has been done with a white light stimulus only and must be extended to a stimulus of varying colour before these suggestions can be established. It is also possible that, instead of rods, 'blue' cones are responsible for after-blueness or that it occurs peripherally because of the greater pooling of response from the receptors here.

It is interesting to compare Polyak's (1941) work on the rod-free area with the present work on the after-blueness-free area. He gives the rod-free area for man as 1° 40′ in diameter and this is shown in Fig. 5*a* together with the areas which just succeed and just fail to produce foveal after-images for B.L. and P.W.T. Figure 5*b* compares Polyak's rod-free area with the retinal positions which just succeed and just fail to produce after-images for the ½° and 1° fields for observer B.L. In all cases when after-images are not seen, there are no rods and when they are seen, rods are present (shown shaded). The shape of the after-image, however, is always a complete disk like the stimulus and is not related to rod distribution. The work should also be mentioned of Østerberg (1935) who found the rod-free area as 45′ instead of Polyak's 1° 40′. However,

it is probable that the density of rods between these fields is very low, which may partly account for the discrepancy between Polyak's and Østerberg's results. Figure 5 should be regarded as showing that the rod-free area and after-blueness-free area are of the same order of magnitude rather than showing an exact agreement, as undoubtedly rod density plays a part. In any case rod density has to be considered to explain P.W.T.'s not seeing after-images at 1° off the fovea. The smaller sensitivity of P.W.T. than that of B.L. may be due less to retinal structure than to less good concentration in the difficult task of extrafoveal observations, where the normal association of fixation and judgement is broken down. The predominant difficulty in this investigation, particularly when the after-effect does not immediately follow the stimulus, is in making a judgement on the particular part of the retina stimulated. For instance, if a field is viewed at, say, 4° off, and the observer searches for an after-effect at 3° off, he may miss the after-effect at 4° off, especially if it is barely visible anyway, and see nothing.

After-blueness following stimuli of all colours is possibly associated with Wright's (1946) 'positive blue' phenomenon. Wright looked at a bright 5° yellow adapting field for 3 min and then at a 2° yellow field which he found to contain an unexpected quantity of blue which disappeared after about 2 min. Moreover, the amount of blue was independent of the brightness of the 2° yellow field, which led him to interpret the phenomenon as a superimposed blue after-image. This was not unexpected after adaptation to yellow; but less expected was a similar blue phenomenon superimposed on the 2° yellow field after blue adaptation. This may be better understood in view of the above if it is a rod phenomena. But, as Wright points out, after-images are not the full explanation, as no blue after-image occurs simply after viewing the adapting light for 3 min. Perhaps there is some interaction between a subliminal after-image and the 2° field.

In the second group the effects are detailed on pp. 72, 75. From their appearance the 'light' after-effects suggest an excitatory origin and the 'dark' after-effects an inhibitory origin. Continuous after-blueness may be excitatory, after-blackness inhibitory and intermittent after-blueness an alternation of excitatory and inhibitory processes. These may possibly be indirectly associated with 'on' and 'off' effects respectively.

SUMMARY

1. The after-effects of a white light stimulus, subtending $\frac{1}{2}$°–5°, lasting $\frac{1}{2}$–64 sec, of retinal illumination $\bar{3}$·2–5·7 log. trolands, and applied foveally or up to 5° off the fovea, can be classified as 'light' or 'dark' after-effects.

2. 'Light' after-effects are those where the field is apparently replaced by an area of the same size and shape as the stimulus and brighter than the

surroundings. 'Light' after-effects are subdivided into persistence of vision, after-colours and after-blueness (continuous or intermittent). Because of its different nature persistence of vision has not been investigated.

3. After-colours (meaning a long-lasting coloured after-effect with many changes of colour) occur both foveally and peripherally for all field sizes for retinal illuminations above 4·8 log. trolands. This phenomenon is probably associated with cones.

4. After-blueness (meaning a shorter after-effect of a bluish colour) occurs from about 1·3 to 4·8 log. trolands. It is seen both foveally and peripherally for the 2° field and larger but only peripherally for a 1° or $\frac{1}{2}$° field. It can be continuous or intermittent. Continuous after-blueness stays at the same intensity throughout and occurs for short stimulus times only, often 4 sec or less. In intermittent after-blueness several distinct images are seen, each one lasting and separated from the next one by about a second; it has a higher threshold than continuous after-blueness.

5. After-blackness (meaning an effect where the field is apparently replaced by an area of the same size and shape as the stimulus but darker than the surroundings) can occur as single or as several separate images. It can follow a stimulus on its own or after any of the 'light' after-effects. It occurs for long stimulus times only, often 2 sec and more. With a 1° field it is present foveally and peripherally.

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PLASMA ADRENALINE AND NORADRENALINE DURING DIFFUSION RESPIRATION

By R. A. MILLAR

From the Department of Anaesthesia, Montreal Neurological Institute and McGill University, Montreal, Canada

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Draper & Whitehead (1944) showed that dogs maintained in apnoea with large doses of thiopentone could survive for periods of half an hour or longer when exposed to 100% oxygen through a patent airway at atmospheric pressure. The critical factor limiting the duration of this condition, termed 'diffusion respiration', was a severe respiratory acidosis secondary to progressive elevation of alveolar carbon dioxide tension (Draper, Whitehead & Spencer, 1947). Provided that alveolar and tissue nitrogen are at least partially eliminated beforehand, such states of oxygenated apnoea provide an opportunity to study the effects of progressive rises in arterial CO_2 tension, with a minimum of interference from anoxia, and without respiratory stimulation (Holmdahl, 1956). In this study direct measurement has been made of plasma adrenaline and noradrenaline concentrations during diffusion respiration in dogs maintained in apnoea by means of a continuous infusion of succinyl choline.

METHODS

Seven dogs (7–16 kg) were lightly anaesthetized with intravenous thiopentone. The trachea was intubated with a No. 9 Magill cuffed portex tube, this being connected through a T-piece to an expiratory valve and a 5 l. rubber bag, which was kept partly filled by a flow of 100% oxygen. The femoral artery on one side was cannulated after infiltration of the groin with 5–10 ml. of 1% lignocaine solution; arterial blood pressure was measured continuously by means of a Statham strain gauge (Model p 23–A), Sanborn amplifier and recorder; a three-way tap allowed withdrawal of blood samples as required. Heparin 10,000 u. was injected intravenously.

The dogs were allowed to breathe 100% oxygen for 20–30 min, after which spontaneous respiration was abolished by means of a continuous intravenous infusion, containing 200 mg succinyl choline chloride in each 100 ml. of 0·9% (w/v) sodium chloride solution, which was given at a very slow rate throughout each study. Before the start of diffusion respiration the dogs were ventilated manually for 10 min with 100% oxygen, using high gas flows to obtain good oxygenation with adequate elimination of nitrogen and carbon dioxide. The first arterial blood sample for assay was withdrawn during this period of ventilation. Manual respiration was then stopped and the endotracheal tube left attached to the gas bag which was kept partly filled with 100% oxygen. The expiratory valve was loose to ensure that no positive pressure was applied to the airway.

In two of the seven studies blood samples were withdrawn during diffusion respiration at irregular time intervals up to 79 min. In the other five experiments samples were withdrawn for assay after 15, 30 and 60 min of apnoea. After each blood sample was withdrawn an equal volume of 0·9% (w/v) sodium chloride solution was injected intravenously.

The period of diffusion respiration was terminated by manual overventilation with 100% oxygen. In two studies blood samples were withdrawn 1–2 min after ventilation was started, and in all seven experiments a sample was taken after 10 min. The endotracheal tube was then disconnected from the oxygen supply, and apnoea was continued with the airway open to air, a final blood sample being withdrawn 7–9 min later, just before cardiac arrest occurred from acute asphyxia.

Blood samples for assay of adrenaline and noradrenaline (35 ml.) were withdrawn into glass tubes containing a few drops of heparin (10,000 u./ml.), and were centrifuged immediately. The plasma was aspirated, filtered, and applied to alumina columns. Adrenaline and noradrenaline in the plasma eluates were estimated fluorimetrically by the trihydroxyindole method, which has been described previously (Millar, Keener & Benfey, 1959); an error of $\pm 25\%$ is involved in the differential estimation of adrenaline and noradrenaline in single plasma samples. The values, which refer to μg free base/l. of plasma, are uncorrected for losses in recovery up to 30%.

Blood for pH, pCO_2, and 'standard' bicarbonate estimations (8 ml.) was withdrawn anaerobically into syringes moistened with heparin. Whole-blood pH, and the pH of separated plasma equilibrated at 38° C with a known CO_2 tension (40 mm Hg), were determined at once, using the 'Radiometer' pH meter and Astrup apparatus (Astrup & Schrøder, 1956; from these two pH determinations the 'standard' bicarbonate in separated plasma, and the plasma CO_2 tension, were calculated as described by Astrup (1956); in three studies arterial oxygen saturation was determined spectrophotometrically (Nahas, 1951).

RESULTS

Changes in arterial pCO_2 and pH

Diffusion respiration resulted in a progressive respiratory acidosis, shown by an increase in arterial pCO_2, a declining pH, and a linear slope of the (semi-log) plot of pCO_2/pH. Table 1 shows the values in a typical experiment, in which a pCO_2 of 390 mm Hg and a pH of 6·47 were reached after 60 min of diffusion respiration. Similar results were obtained in another four experiments, in which apnoea was continued for the same period of time; the average values in this group of five studies have been included in Table 4. After 15 min of diffusion respiration CO_2 tensions of 89–126 mm Hg were reached, at 30 min the range was 120–266 mm Hg, and by 60 min the levels were 310–390 mm Hg. The maximum pCO_2 reached in any experiment was 427 mm Hg, at a pH of 6·48, after 79 min of apnoea.

The values for 'standard' bicarbonate are based on equilibration of separated plasma with a pCO_2 of 40 mm Hg and represent, essentially, the non-respiratory component of acid–base balance (Jørgensen & Astrup, 1957). Although small increases occurred at variable times during diffusion respiration, the 'standard' bicarbonate concentration did not exceed 24 m-mole/l. in any experiment, and in general the changes were rather inconsistent. Because of initial over-ventilation the CO_2 tension in several experiments was low (Table 4), and the rise in pCO_2 during the first 15 min of diffusion respiration

was accompanied by a rise in 'standard' bicarbonate. This early increase in 'standard' bicarbonate did not occur in the three experiments in which pCO_2 levels were somewhat higher (34, 39 and 42 mm Hg).

TABLE 1. Changes during one experiment in whole-blood pH, plasma pCO_2, and 'standard' bicarbonate content of plasma, during 1 hr of diffusion respiration (D.R.), and after the subsequent 10 min of ventilation with 100% oxygen

	Time (min)	pH	pCO_2 (mm Hg)	'Standard' bicarbonate (m-mole/l.)
Control	—	7·36	42	22
D.R.	+15	6·96	118	22
D.R.	+30	6·67	266	23
D.R.	+60	6·47	390	20
Ventilation, O_2	+10	7·31	28	14

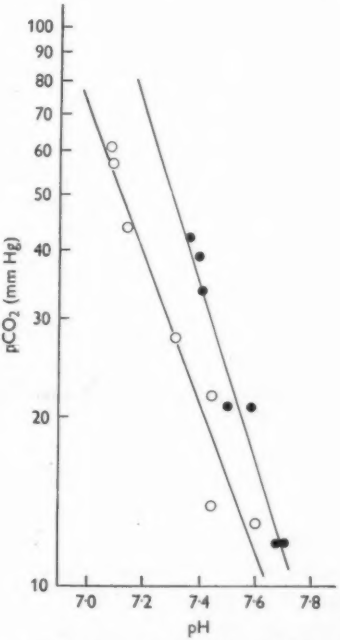

Fig. 1. Graphs of pCO_2/pH for blood samples withdrawn before (●) and after (○) diffusion respiration in seven experiments. The plot for samples obtained after diffusion respiration is shifted to the left, indicating the existence of a non-respiratory acidosis. Semi-log. scale.

Ventilation with 100% oxygen, after 1 hr or longer of diffusion respiration, brought about a rapid fall in arterial pCO_2, as is shown by the low tensions measured after 10 min of ventilation (Tables 1 and 4). In one experiment a fall of 220 mm Hg occurred within 2 min of starting ventilation, the decline in CO_2 tension being exponential rather than linear (Table 5).

Average pCO_2 and 'standard' bicarbonate before diffusion respiration, in the seven experiments, were 29 mm Hg and 19 m-mole/l., whereas after diffusion respiration the values were 38 and 14 respectively. Although the average pCO_2 was higher, the 'standard' bicarbonate was significantly less ($P < 0.01$). Figure 1 shows the pCO_2/pH plots for blood samples withdrawn before diffusion respiration, compared to the blood samples withdrawn during ventilation after diffusion respiration. The shift to the acid side of the pCO_2/pH line shows, as does the fall in 'standard' bicarbonate noted above, that a non-respiratory acidosis existed after the period of dif-

TABLE 2. The gradual decline in oxygen saturation (%) measured in three experiments during diffusion respiration (D.R.)

Sample	Time (min)	1	2	3
Control	—	99	—	99
D.R.	+15	—	—	98
D.R.	+30	89	100	97
D.R.	+60	87	92	94
Ventilation, O_2	+10	100	—	101

TABLE 3. Asphyxial changes resulting in one experiment when apnoea (subsequent to the period of diffusion respiration) is maintained with the airway open to atmospheric air

	pH	pCO_2 (mm Hg)	'Standard' bicarbonate (m-mole/l.)	O_2 saturation (%)
Control	7·60	13	15	100
Asphyxia +8 min	6·95	98	18	30

fusion respiration had ended. These findings agree with previous evidence that a metabolic acidosis is produced by diffusion respiration (Joels & Samueloff, 1956*a*).

In the three experiments in which measurements were made, a slow fall in arterial oxygen saturation occurred during diffusion respiration. This is illustrated in Table 2.

Table 3 shows the changes in blood gases 8 min after disconnecting the oxygen supply in one experiment. In this and the other studies cardiac arrest resulted from acute anoxia after only 8–11 min of apnoea when the airway was open to atmospheric air, instead of the 100% oxygen available during diffusion respiration.

Plasma adrenaline and noradrenaline

Increases in the plasma concentrations of adrenaline and noradrenaline occurred throughout diffusion respiration. The average values in five experiments are shown in Table 4. In the control samples, withdrawn during the period of ventilation which preceded diffusion respiration, plasma noradrenaline and adrenaline were in the ranges 0–1·1 and 0–0·42 μg/l. respectively. After

15 and 30 min of apnoea noradrenaline levels were 0·30–2·0, and 1·0–5·7 μg/l. respectively. Plasma adrenaline concentrations were in the range 0·04–1·1 after 15 min and 0·85–6·0 μg/l. after 30 min of apnoea. When diffusion respiration had continued for 60 min adrenaline levels were in the range 12·8–30·4 μg/l., plasma noradrenaline being much lower, 3·3–7·7 μg/l. Figure 2 illustrates a typical study in which plasma noradrenaline showed the greater rise during the first 30 min of apnoea; after 60 min adrenaline was greatly increased.

TABLE 4. Average increases in five experiments in plasma adrenaline and noradrenaline concentrations, with arterial blood pressure changes, induced by respiratory acidosis during 1 hr of diffusion respiration (D.R.). The effects of the subsequent 10 min period of ventilation with 100% oxygen are also shown

	Time (min)	pH	pCO_2 (mm Hg)	'Standard' bicarbonate (m-mole/l.)	Adrenaline (μg/l.)	Noradrenaline (μg/l.)	Arterial B.P. (mm Hg) systolic	Arterial B.P. (mm Hg) diastolic
Control	—	7·56	22	18	0·14	0·38	167	120
D.R.	+15	7·01	108	23	0·59	1·2	178	87
D.R.	+30	6·82	173	21	2·6	3·1	238	126
D.R.	+60	6·55	338	22	18·7	4·9	120	64
Ventilation, O_2	+10	7·39	24	14	1·1	0·65	123	94

Ventilation induced a profound and rapid fall in plasma catecholamine concentration, which reached low levels after 10 min or earlier (Table 4 and Fig. 2). The rapidity of this decline was demonstrated clearly in two experiments in which blood samples were withdrawn 2 min after the start of ventilation. In one experiment plasma adrenaline and noradrenaline had already fallen from a total level of 18·6 to 8·0 μg/l. of plasma; the results in the other study were similar and are illustrated in Table 5.

The effect of acute asphyxia on plasma adrenaline and noradrenaline is shown in Table 6. In all seven experiments the levels of both amines increased greatly, as expected (Houssay & Molinelli, 1926).

Blood pressure

Alterations in blood pressure were quite consistent during diffusion respiration. Table 4 shows the average values during 60 min of apnoea in five experiments. A fall in diastolic pressure, with a smaller and less constant increase in systolic pressure, was a characteristic feature in the first 15 min of diffusion respiration. Pronounced rises, mainly of systolic pressure, then reached a broad plateau centred around the 30 min period. A gradual decline occurred subsequently and the average mean arterial pressure level after 60 min of apnoea was 53 mm Hg below that before diffusion respiration. During one more prolonged study in which the rise in pCO_2 was unusually slow (circulating adrenaline and noradrenaline levels being lower than in the

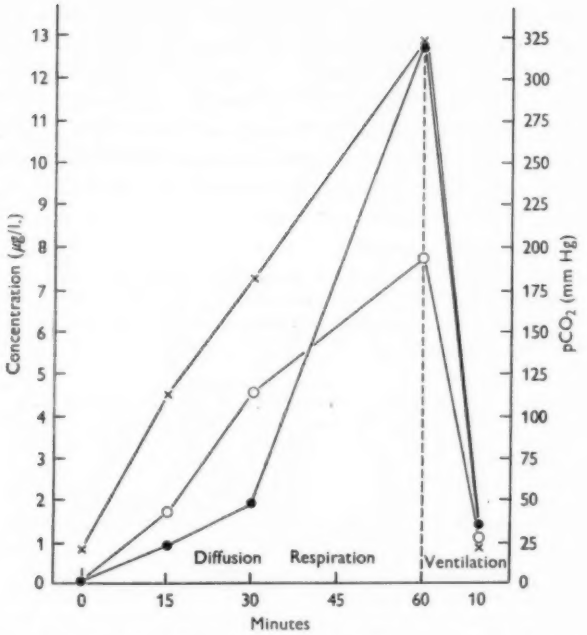

Fig. 2. Increases in plasma adrenaline (●) and noradrenaline (○) concentrations as the arterial pCO_2 (×) rises during diffusion respiration, in a typical experiment. Also shown are the rapid falls in CO_2 tension and concentrations of circulating catecholamines which occur when diffusion respiration is terminated by ventilation with 100% oxygen.

TABLE 5. The effects in one experiment of 2 min of ventilation with 100% oxygen on plasma adrenaline and noradrenaline concentrations and on the severe respiratory acidosis induced by prolonged diffusion respiration (D.R.)

Sample	Time (min)	pH	pCO_2 (mm Hg)	'Standard' bicarbonate (m-mole/l.)	Adrenaline (μg/l.)	Noradrenaline (μg/l.)
D.R.	+79	6·48	427	23	31·9	15·5
Ventilation	+ 2	6·66	207	17	9·4	3·5
O_2	+10	7·09	57	16	0·63	1·9

TABLE 6. The effect of asphyxia, induced by maintaining apnoea with the airway open to air, on plasma adrenaline (A) and noradrenaline (N) levels (μg/l.) in seven dogs. The control samples were withdrawn after the 10 min period of ventilation which followed an hour or longer of diffusion respiration; the 'control' values of plasma adrenaline and noradrenaline concentrations are therefore higher than those measured under basal conditions

	1		2		3		4		5		6		7	
	A	N	A	N	A	N	A	N	A	N	A	N	A	N
'Control'	0·63	1·9	1·1	1·3	0·64	0·72	1·4	1·1	0·69	0·36	1·5	0·19	1·4	0·86
Asphyxia, 8–10 min	11·8	14·2	13·3	14·6	14·9	18·9	8·6	5·1	3·3	8·2	66·8	14·5	9·5	4·3

other experiments), mean arterial blood pressure after 77 min of apnoea was still 32 mm Hg above the pre-diffusion respiration value.

When manual ventilation was begun a steep rise in blood pressure followed almost immediately, as is shown in Fig. 3(*a*), the average systolic and diastolic increase in seven experiments amounting to 94 mm and 25 mm Hg respectively. A gradual fall then occurred and after 10 min of ventilation average mean arterial blood pressure in five experiments was 32 mm Hg below that measured before diffusion respiration.

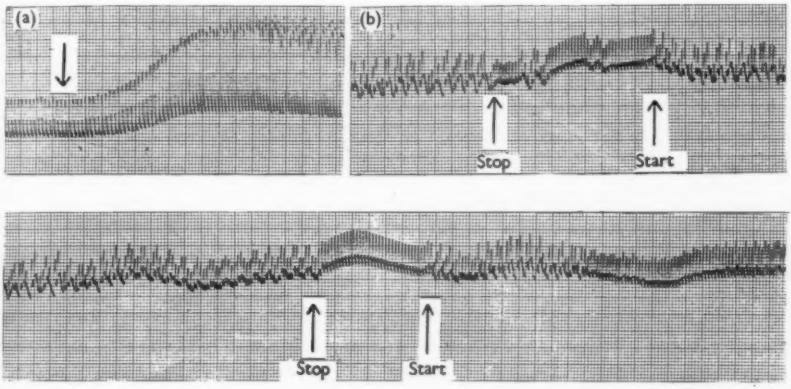

Fig. 3. *a*, The effect on arterial blood pressure of ventilation with 100% oxygen (started at arrow), after 1 hr of diffusion respiration. Each large division on the record represents 2 sec. *b*, Cardiac arrhythmias observed on the blood-pressure tracing during the period of ventilation which followed diffusion respiration.

Cardiac arrhythmias, during ventilation after diffusion respiration, were observed on the blood pressure record in only two out of seven experiments. In one the appearance and disappearance of arrhythmia appeared to bear a direct relationship to lung inflation, as is shown in Fig. 3(*b*); and in the other experiment cardiac irregularities persisted for only 12 sec after ventilation was finally stopped.

Asphyxia resulted in a progressive rise in systolic and diastolic pressures, terminating in gross hypertension before an abrupt down-swing, with bradycardia, preceded cardiac arrest.

DISCUSSION

These experiments show that increases in the plasma concentrations of adrenaline and noradrenaline accompany the respiratory acidosis produced by diffusion respiration, and that when the arterial CO_2 tension is lowered by ventilation circulating catecholamines are also lowered to near-basal levels.

It has not been established whether the changes in plasma amine concentration are induced by the increased arterial pCO_2 or by the reduction in blood pH, but liberation of catecholamines during respiratory acidosis must result largely from stimulation of central sympathetic centres (Mathison, 1911), either directly or by reflex chemoreceptor drive. This may be reinforced by various peripheral mechanisms, resulting from the effects of a shift of pH to the acid side, from the rise in plasma potassium induced by carbon dioxide (Young, Sealy & Harris, 1954), and from interference with the metabolic transformation of catecholamines. Since the amounts of adrenaline occurring outside the adrenal medulla are small, marked increases in plasma adrenaline can only result from adrenal medullary liberation. For example, the pronounced rise in circulating adrenaline during severe haemorrhage in dogs (Millar & Benfey, 1958) is almost entirely abolished by bilateral adrenalectomy (Millar *et al.* 1959). It can be assumed, therefore, that the increased adrenaline levels after 60 min of diffusion respiration result from adrenal medullary secretion, which probably also plays a major part in the pronounced rise in circulating noradrenaline at this later stage.

The mechanisms involved in the early rise in noradrenaline are more conjectural, because of the possibility of noradrenaline liberation from the adrenal medulla and at sympathetic nerve endings. It is not yet certain whether noradrenaline liberation at sympathetic nerve endings can bring about greater rises in plasma noradrenaline than would be induced by simultaneous adrenal medullary discharge, or whether adrenergic nerve endings can be stimulated without simultaneous excitation of the adrenal medulla. The amount of noradrenaline which can be liberated from sites outside the adrenal medulla in response to strong, probably maximal, sympathetic stimulation was shown by recent experiments, however, in which it was found that in exsanguinated, adrenalectomized dogs the maximum plasma noradrenaline level reached was 0·7 μg/l., whereas in the presence of adrenergic blockade by dibenzyline levels up to 4·1 μg/l. were measured under similar conditions. This indicates that plasma noradrenaline levels above 1 μg/l. could be derived either from the adrenal medulla or from adrenergic receptors at which there is delayed destruction of liberated transmitter substance. The possibility cannot be discounted that a reduction in tissue pH or a local effect of carbon dioxide at adrenergic receptor sites allows an accumulation of noradrenaline liberated there as a result of increased central sympathetic discharge; the mechanism could be similar to that attributed to dibenzyline by Brown & Gillespie (1957).

In previous experiments performed in this laboratory on intact dogs, consistent increases in plasma noradrenaline have not been measured without rises in adrenaline which were as great or greater. This suggests that the initial effects of respiratory acidosis are different from those of haemorrhagic hypotension, for instance, in which increases in plasma adrenaline greatly

exceed those of noradrenaline even from the earliest stage (Millar & Benfey, 1958).

Central vasomotor discharge, resulting from brain stem activation by high tensions of arterial CO_2 (Joels & Samueloff, 1956*b*), and shown on the blood pressure record as rhythmic 'waves', disappeared before catecholamine levels reached a maximum, which again suggests that plasma adrenaline and noradrenaline concentrations are increased during diffusion respiration beyond levels induced solely by stimulation of efferent sympathetic nerves.

Comparison of the increased plasma adrenaline levels during diffusion respiration with those measured previously during graded haemorrhagic hypotension (Millar & Benfey, 1958), shows that, when circulatory collapse from haemorrhage was imminent, mean arterial blood pressure (average in eight dogs) was 21 mm Hg and plasma adrenaline was 19 μg/l. Identical circulating adrenaline levels (average in five dogs) were measured after 60 min of diffusion respiration, but the mean arterial blood pressure was 83 mm Hg, indicating that hypotension is not the principal factor concerned in the high adrenaline levels in the later stages of diffusion respiration.

After 60 min of apnoea, blood loss due to withdrawal of samples for assay amounted to 180 ml. in five identical experiments, equivalent to an average loss of 22 ml./kg. It was shown in a previous study (Millar & Benfey, 1958) that blood loss of 24 ml./kg was accompanied by plasma adrenaline levels in the same range as those estimated when diffusion respiration had been terminated by ventilation (noradrenaline levels were much lower in haemorrhage). Although basal catecholamine levels were not regained after diffusion respiration, the pronounced fall in circulating adrenaline and noradrenaline which occurred when respiratory acidosis was reversed shows that blood loss due to sampling was not an important factor in raising plasma adrenaline levels during the apnoeic state. However, the gradual reduction in oxygen saturation after periods of diffusion respiration longer than about 30 min implies that anoxia is partly responsible for the increased circulating catecholamine levels in the later stages of diffusion respiration.

The occurrence of a metabolic acidosis in diffusion respiration, which has been confirmed in these experiments, can be partly accounted for by a raised level of circulating lactate (Joels & Samueloff, 1956*a*). Intravenous infusions of adrenaline have been shown to increase blood lactate concentration in dogs (Brewster, Isaacs, Osgood & King, 1956), so that a close relationship probably exists between the increases in plasma adrenaline and the metabolic acidosis of diffusion respiration.

Studies of the complex circulatory changes accompanying diffusion respiration have produced conflicting results. Holmdahl (1956), in reviewing these, has emphasized the complicating factors introduced when apnoea is maintained by large doses of intravenous barbiturates or by tubocurarine. Succinyl

choline seems preferable in this respect, because it produces neuromuscular paralysis in doses many times less than those required to block autonomic ganglia (Thesleff, 1952).

The consistent fall in diastolic pressure seen during the first 15 min of diffusion respiration in these experiments might be attributable to vasodilation induced by carbon dioxide. However, the evidence for a direct vasodilator action of carbon dioxide was first obtained by carbonic acid perfusion experiments in which extremely high tensions of carbon dioxide were used (Bayliss, 1901), so far outside the physiological range that the evidence seems insecure (Bernthal & Shoemaker, 1937). The increase in plasma adrenaline which occurred over the same period of time, although moderate, could be largely responsible for the lowered diastolic pressure during the early phase of diffusion respiration; such an effect may be related to increased production of lactic acid (Lundholm, 1957).

The raised mean arterial blood pressure noted after about 50 min of apnoea was due almost entirely to a marked increase in systolic pressure. Although diastolic pressure rose to a variable degree during the second 15 min of apnoea, the average level at 30 min was only slightly higher than the control value. These findings suggest that cardiac output was increased, as a result of direct myocardial stimulation (Boniface & Brown, 1953) or from central sympathetic excitation. The depressant effects of carbon dioxide were subsequently demonstrated by a fall in arterial blood pressure, in spite of the greatly increased levels of circulating catecholamines.

Adverse circulatory effects have been reported as a result of rapid reduction of elevated CO_2 tensions. Foremost among these is the occurrence of ventricular fibrillation when dogs are allowed to breathe air after inhaling mixtures of 30–40% carbon dioxide in oxygen for several hours (Brown & Miller, 1952). Because of the high alveolar pCO_2 present initially during such a period of ventilation it seems certain that the introduction of an air atmosphere would lead to arterial oxygen desaturation, which could readily explain a high incidence of serious cardiac arrhythmias. In the experiments described here ventilation with 100% oxygen lowered even higher arterial pCO_2 tensions at very rapid rates, without inducing ventricular fibrillation. Another factor of possible relevance to the occurrence of cardiac arrhythmias would be a sudden change in plasma adrenaline and noradrenaline levels, and it has been reported, on rather indirect evidence, that the concentration of circulating catecholamines increases abruptly as an elevated arterial pCO_2 is lowered (Tenney, 1956). The findings obtained here do not support the occurrence, on starting ventilation, of further transient increases in the concentration of plasma catecholamines to a level above that already measured after prolonged diffusion respiration, since a pronounced fall in the levels of circulating adrenaline and noradrenaline was found to occur as early as 1 min after ventilation

with oxygen was begun. The sudden increase in blood pressure noted immediately after ventilation started, on the other hand, does indicate an increase in cardiovascular reactivity, in association with the rise in pH or the fall in CO_2 tension. Whether this is a vascular or a cardiac change is uncertain, but an increase in plasma potassium (Young *et al.* 1954) and in cardiac contractile force (Boniface & Brown, 1953) have been shown to occur when high carbon dioxide concentrations are lowered.

SUMMARY

1. Rises in arterial CO_2 tension were induced by maintaining lightly anaesthetized dogs in a state of oxygenated apnoea, or diffusion respiration, by means of a succinyl choline infusion; a pCO_2 of 427 mm Hg was the most extreme level reached, at an arterial pH of 6·48.

2. Plasma adrenaline and noradrenaline concentrations, estimated by the trihydroxyindole method, showed progressive increases during diffusion respiration. In the first 30 min the rise was predominantly in noradrenaline; subsequently plasma adrenaline increased greatly, reaching levels up to 30 μg/l. after 60 min of apnoea.

3. Ventilation rapidly reduced the arterial CO_2 tension and brought circulating catecholamine concentration to near-basal levels. There was no evidence of a further increase in plasma adrenaline and noradrenaline when ventilation started, nor did untoward cardiac effects occur during reduction of arterial pCO_2.

4. When CO_2 tension had been lowered a shift to the acid side of the plot of pCO_2/pH and a reduction in 'standard' bicarbonate demonstrated the existence of a non-respiratory acidosis.

5. Diastolic blood pressure was reduced during the first 15 min of diffusion respiration; thereafter marked increases in systolic pressure occurred. After 1 hr of diffusion respiration blood pressure was depressed, but ventilation produced an immediate, pronounced, but temporary rise in blood pressure.

I wish to thank Mrs M. Laidlaw and Dr J. Bidzinski for their assistance during these studies.

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SINGLE UNIT ACTIVITY IN LATERAL GENICULATE BODY AND OPTIC TRACT OF UNRESTRAINED CATS

By D. H. HUBEL*

From the Department of Neurophysiology, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington 12, *D.C., U.S.A.*

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In two recent studies of the cat's striate cortex (Hubel, 1959; Hubel & Wiesel, 1959) single units were shown to react to light stimuli in a highly specific manner. Most units responded either feebly or not at all to stimulation of the retina with diffuse light, but gave brisk responses to stationary or moving restricted spots of light. Responses to moving spots often varied with the direction of movement. It was clear that such responses must be the result of complex integrative mechanisms.

The present study was undertaken to find out whether similar responses occur in retinal ganglion cells or cells of the dorsal lateral geniculate body. Lateral geniculate units have not previously been studied with restricted light stimulation, and although the cat's retinal ganglion cell has been extensively investigated by Kuffler and his co-workers (Kuffler, 1953; Kuffler, FitzHugh & Barlow, 1957), responses to moving spots were not examined. Thus it has not been possible to say whether the complex activity of cortical units originates in the cortex itself, or at lower levels.

Methods for stereotaxic depth recordings in the unanaesthetized unrestrained animal were developed in order to make cortical and depth studies under similar conditions. These techniques make it possible to record from single units from virtually any part of the brain of the freely moving animal.

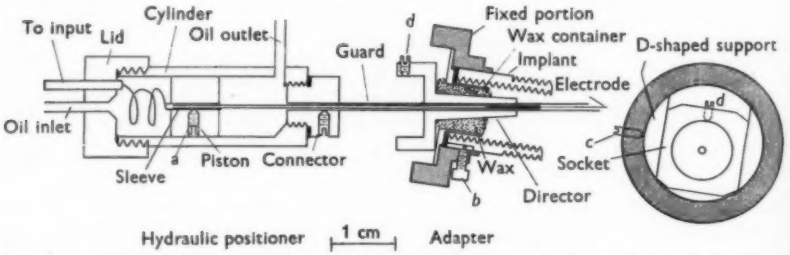
METHODS

For depth recording of single units in the unrestrained animal, a tungsten micro-electrode was advanced by a hydraulic micro-electrode positioner (Text-fig. 1). The positioner was attached to a chronically indwelling plastic implant during recording and removed between recordings. An adjustable adapter connected the positioner to the implant and allowed the direction of penetration to be varied over a wide angle. A Horsley–Clarke stereotaxic instrument was used in adjusting the adapter to a pre-assigned target in the brain. With this method it was possible to

* Present address: Neurophysiological Laboratory, Department of Pharmacology, Harvard Medical School, 25 Shattuck Street, Boston 15, Massachusetts.

record from single units in the depths of the brain for periods of several hours, despite movements of the head of the freely moving cat.

The technical details given in the following paragraphs are not necessary for an understanding of the results to be presented. The electrode positioner was similar in principle to that used for cortical recording (Hubel, 1959); a piston was raised or lowered inside a Perspex cylinder by mineral oil conveyed through a vent in the cylinder's lid. A fine (26-gauge) hollow steel needle mounted in the piston held the electrode in its lower end. This electrode holder was clamped in a metal sleeve by a set screw (Text-fig. 1*a*), and was easily replaced by steel needles of other lengths. The metal sleeve was joined to the input pin in the lid of the cylinder by a coiled wire.



Text-fig. 1. Diagram of micro-electrode positioner, adapter, and implant. Hydraulic positioner and adapter are shown in cross-section; a top view of the adapter is shown to the right of the figure. Fixed portion of the adapter is cross-hatched. Set screws (*a–d*) are described in the text.

A brass connector, threaded into the cylinder's lower end, attached the hydraulic positioner to the adapter. The connector held a steel guard, a 19-gauge hollow steel needle sharpened at its lower end. The 26-gauge electrode holder could slide freely inside the guard, and was separated from it by a thin insulating coating of Kel-F (fluorocarbon polymer made by Minnesota Mining and Manufacturing Co., Ltd.). Before the beginning of a recording the electrode, bent at its upper end to hold it in position, was inserted into the lower end of the holder. The piston was then retracted until the electrode tip was just inside the guard. When the positioner was attached to the implant, the guard was directed into the brain, its tip coming to rest within several millimetres of the target structure (e.g. the lateral geniculate body). The electrode was then hydraulically advanced over the remaining distance.

The implant was a hollow Kel-F peg 13 mm in diameter and 15 mm long. Its lower half was threaded outside with oversize threads for insertion into the skull (Hubel, 1959). The upper half was smooth except for a single longitudinal slot. The adapter fitted over the outside of the implant and was held by a set screw (Text-fig. 1*b*) which fitted into the slot. Threads lining the inside of the implant held a plug for blocking the hole when recordings were not being made.

The adapter consisted of a fixed part which fitted on the implant (Text-fig. 1, shaded portion), and an adjustable socket and director which held the micro-electrode positioner. The guard was inserted through the director until the lower part of the brass connector came to rest in the socket. The outside of the socket formed a rectangular block (with bevelled corners). Two D-shaped supports held this block firmly between them. The supports fitted into the cup-shaped upper recess of the fixed portion of the adapter. They could rotate in this recess, and the socket and director could tilt and move back and forth between them. The angle between micro-electrode positioner and implant was thus variable over a wide range, being limited only by the inside wall of the implant. Once adjusted, the position of the socket and director was fixed by a single set screw (Text-fig. 1*c*) which tightened the D-supports against the socket.

For stable recordings a 'closed chamber' was used to seal off the surface of the brain from the atmosphere (Davies, 1956). The space around the director was filled with wax. A thin-walled

brass tube acted as a wax container, keeping the wax within the lumen of the implant. A flange, continuous with the upper end of the tube, was held in position between the floor of the fixed part of the adapter below and the D-shaped supports above. The space above the cortex was completely closed when the upper surface of the implant pressed against a rubber washer beneath the flange.

Before mounting the adapter, the lumen of the implant was filled with oil. The adapter, previously adjusted for a specific target, with wax in place, and with a stilette temporarily blocking the lumen of the director, was placed over the implant, excess oil escaping along the slot. The set screw (Text-fig. 1*b*) was tightened in the slot. The stilette was removed, and the cylinder was attached by inserting the guard into the director and driving its tip carefully into the brain. The cat showed no distress during this procedure. The connector was tightened firmly in its socket by a set screw (Text-fig. 1*d*).

A system was developed for adjusting the adapter, and hence the aim of the needle entering the brain. The anaesthetized animal was put in the stereotaxic apparatus only for the initial implantation. The implant was mounted in the skull by the method described for cortical recordings (Hubel, 1959). Its precise position and orientation were not critical: most were placed 19 mm lateral to the mid line, at Horsley–Clarke frontal plane + 6·5 mm. From this site both the left and the right lateral geniculates could be reached.

A brass tube served as a substitute for the implant. Its upper half was of the same shape, slotted in the same manner, as the upper part of the implant. Its lower half was wider, and fitted smoothly over the implant. A set screw in the lower section fitted into the slot of the implant, so that when the substitute peg was placed over the implant the slots of both were in line. The substitute peg was mounted on a Horsley–Clarke electrode carrier in such a way that advancing the electrode carrier moved the peg along its own axis. After the implant was inserted the electrode carrier was adjusted so that when advanced the substitute peg fitted precisely over the implant (Plate 1*a*). Thus when the animal was removed from the stereotaxic instrument the electrode holder had only to be advanced a further known distance to bring the substitute peg into the implant's former position.

The adapter was adjusted by placing it on the substitute peg, positioned as described above, and by setting a second electrode carrier to indicate the position of the target (Plate 1*b*), as determined from a stereotaxic cat atlas (Jasper & Ajmone-Marsan, 1954). A 19-gauge stilette was put through the director, and the adapter adjusted so that the stilette's tip coincided with the target. The D-shaped supports were then tightened with set screw *c* (Text-fig. 1), fixing the socket and director in position. The distance from the floor of the socket to the target was measured, for determining the lengths of the electrode holder and guard. The adapter was removed from the substitute peg. For the closed chamber, melted wax was dripped into the space inside of the wax container, so as to surround the director. When these adjustments resulted in successful recordings from the lateral geniculate, the same settings were kept for several penetrations, and then changed slightly to avoid making many penetrations along the same track.

The micro-electrodes were electropolished tungsten wires coated with a vinyl lacquer (Hubel, 1957*b*). Electrolytic lesions were made by a method described previously (Hubel, 1959).

Methods for visual stimulation were generally the same as those used in cortical studies (Hubel, 1959). A large screen several feet from the cat was illuminated diffusely or by a circular spot of light subtending an angle of 2° at the cat's eyes (corresponding to 0·5 mm on the cat's retina). The spot luminance measured 1·0 cd/m^2, and was always used against a diffuse background light, having a luminance 1·9 $\log_{10}$ units less, to avoid the greatly increased sensitivity to scattered light which accompanies dark adaptation. On several occasions an annular light stimulus was projected on the screen in place of the usual circular spot. It was of the same brightness, and had an outer diameter subtending 6° at the cat's eyes, and an inner diameter of 2°. It was made by a separate flashlight mounted beside the one producing the spot, adjusted so that both spot and annulus were always concentric.

RESULTS

Criteria for distinguishing cell spikes and axon spikes

In recordings from visual pathways, from optic tract to striate cortex, two types of unit spikes were seen. Spikes of the first type were recorded only from grey matter, and were therefore presumed to be from cells. When first detected they were small (several hundred microvolts) and either negative or negative–positive. As the electrode advanced they became larger (up to several millivolts) and positive–negative, with a clear inflexion on the ascending limb of the positive phase (Text-fig. 2*A*). What was presumed to be synaptic noise was sometimes seen and heard during recordings from units with large spikes.

Text-fig. 2. *A*, Cell-type spike, recorded from cerebral cortex. Note inflexion on rising phase. *B*, Two spikes recorded from optic tract. Note notch immediately following the peak of each spike. Positive deflexions upward; time constant 1 msec; duration of sweeps, *A* 10 msec, *B* 5 msec. Spike amplitudes, *A* 5 mV, *B* 0·2 mV.

Spikes of the second type were recorded from both grey and white matter; in white matter all spikes were of this type. They were presumed to originate from myelinated fibres. They were generally initially positive from the time they were first seen, and were often purely positive. When they first appeared they had no inflexion or notch, and the rising phase was extremely steep. As they reached a maximum of several millivolts and then declined, they usually developed a notch (Text-fig. 2*B*). This varied in position not only from unit to unit, being anywhere on the rising or falling phase, but to some extent from one spike to the next in the same unit. As the electrode advanced the notch gradually deepened until the spike was split into two components, the second of which finally tended to drop out.

In the present studies these criteria are based on anatomical and histological evidence for determining electrode tip positions, white matter giving only fibre spikes, and cell spikes occurring only in grey matter. The criteria are not necessarily valid in regions other than those studied here, and they are probably not infallible even in these areas.

Identification of geniculate units

Histological methods were used to determine the sites from which recordings were made. In Plate 2 an example is given of three electrolytic lesions made

in a single penetration of the right lateral geniculate (shown in frontal section). The electrode penetrated the geniculate body contralateral to the site of implantation, advancing in a slightly dorsoventral direction. The lesion indicated by the left-hand arrow was made to establish a datum point near the beginning of the penetration: a slight advance of the electrode resulted in a marked increase of background unresolved spike activity as the geniculate body was entered. A similar richness of background activity was often found in the cortex, but was not seen in the optic tract or radiations. In the cortex background firing was uninfluenced, or only weakly influenced, by diffuse light stimulation, whereas in the lateral geniculate light flashes produced intense activation. The appearance of background activity easily influenced by diffuse light stimuli seemed to be a reliable indication of the entry of the electrode into the geniculate.

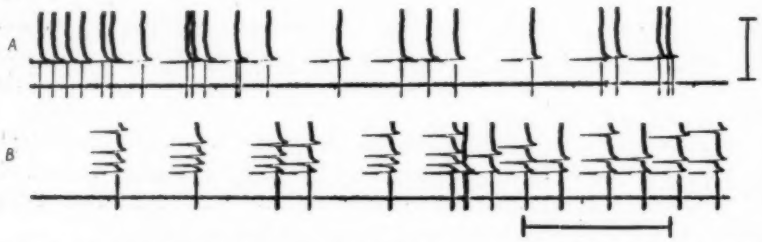
Two more lesions were made in this penetration while recording from single cells within the lateral geniculate nucleus. These are indicated by the middle and right-hand arrows of Plate 2. These units had the characteristic firing patterns and responses to be described below.

In the following paragraphs the term 'geniculate units' refers to lateral geniculate cells or their axons. Units responding to light stimulation and showing the clustered firing patterns to be described were found in the lateral geniculate body, optic radiation, and deeper layers (V and VI) of the striate cortex. Fibres exhibiting these patterns were found at all three levels, whereas cell bodies with this type of activity were seen only in the lateral geniculate nucleus. It is thus likely that these were lateral geniculate units, as defined above, since the principal cells of the lateral geniculate send their axons through the optic radiation to the striate cortex as far as the IVth layer. This conclusion was strengthened by the finding that responses of these units to light were similar at all three levels, but differed greatly from responses of cortical cells (see below).

Patterns of firing of geniculate units: arousal effects and responses to diffuse light

Most geniculate units showed impulse activity in the absence of intentional light stimulation. Striking differences were seen in the pattern of this maintained activity, depending on the animal's waking state. A record of a unit isolated in the lateral geniculate body is shown in Text-fig. 3. In the awake animal (Text-fig. 3*A*) impulses occurred at more or less random intervals, the discharge pattern resembling, at least superficially, that described for retinal ganglion cells by Kuffler *et al.* (1957). Impulses were recorded on moving film as vertical deflexions of an unswept oscilloscope beam, and also as horizontal deflexions of a separate beam swept from below upwards, triggered by the spikes themselves.

As the animal became drowsy and finally slept (Text-fig. 3 *B*), there developed an increasing tendency to firing in characteristic brief, high-frequency clusters of impulses. Each cluster consisted of 2–8 spikes at frequencies of up to 500/sec or more. These are best seen on the expanded time scale of the swept beam. Intervals between spikes were least initially, and increased during the course of each cluster. Clusters occurred at rates of the order of 0·5–5/sec. These bursts of repetitive firing were seldom if ever seen in geniculate units when the animal was alert.



Text-fig. 3. Patterns of firing of a lateral geniculate cell. *A*, cat awake; *B*, cat asleep. Upper beam is swept from below upwards; sweeps are triggered by the spikes. Positive deflexions, upward for continuous bean, to the left for swept beam. Time, continuous beam, 1 sec; swept beam, 10 msec.

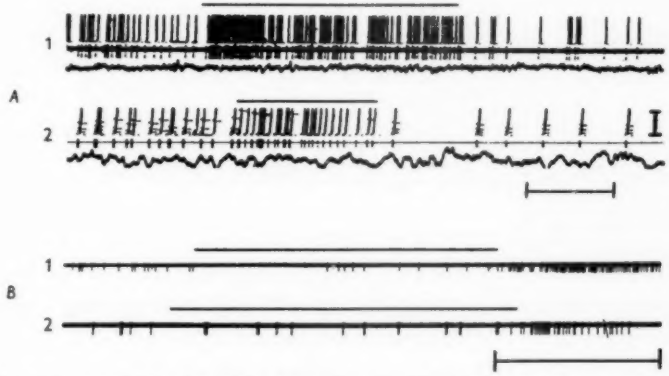
Unlike cortical cells, geniculate units generally responded strongly and consistently to illumination of a large part of the cat's visual field. In sleeping cats responses were studied by stimulation with light diffused through the closed eyelids. Some units gave 'on' discharges to diffuse light stimuli; others responded with 'off' discharges. An occasional unit was activated after both the onset and cessation of the stimulus. The two units illustrated in Text-fig. 4 were recorded from grey matter of the striate cortex or the white matter just beneath. In the unit of Text-fig. 4 *A* a period of illumination lasting several seconds evoked a discharge of impulses. In the upper record (Text-fig. 4 *A*, 1) the animal was awake and alert, as is reflected by the flat surface electrocorticogram in the lower beam. In Text-fig. 4 *A*, 2, the animal was asleep. The surface record showed slow wave activity, and the unit tended to fire in clusters. Light stimulation not only produced a discharge, but also abolished the clustering, whereas during the period of slowest firing following the stimulus, clusters were especially prominent.

The unit of Text-fig. 4 *B* responded with an 'off' discharge following the light stimulus, and during the stimulus period the firing rate was reduced to a rate less than the maintained rate. With the cat asleep clusters were present, and, as in the previous unit, were most common during periods of reduced firing (this time during 'on'), and were abolished when the unit was activated.

Thus light stimuli which in the awake animal activated the unit, had in the

drowsy or sleeping state the additional effect of eliminating clustered activity. Stimuli which decreased firing in the waking animal tended in sleep to increase the number of clusters.

In most experiments some background illumination was present, so that animals could be observed and handled, and also to avoid dark adaptation. As the animal slept, closing of the eyes undoubtedly produced a decrease in retinal illumination. However, passively closing the eyes of a waking cat never produced clusters. In a few experiments done in absolute darkness, arousal by a brief noise had the effect of abolishing clusters. It was therefore clear that the appearance of clusters was not related to a decrease of retinal stimulation resulting from eye closure.



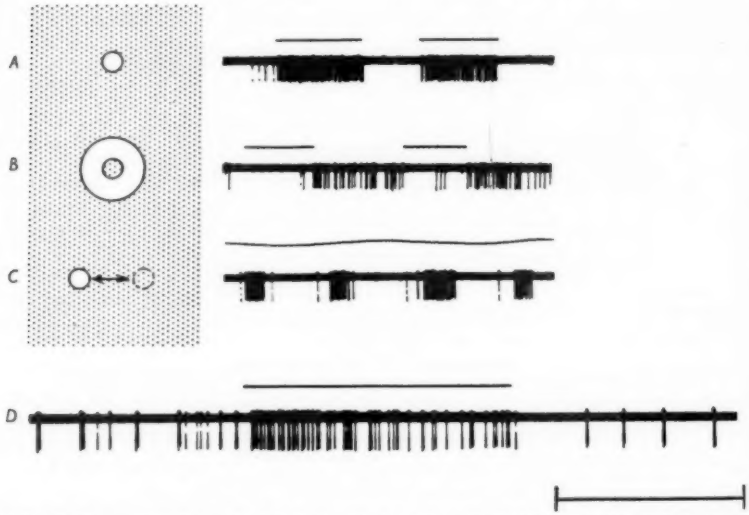
Text-fig. 4. Responses to diffuse light of two fibres recorded from deep in the cortex, possibly from subcortical white matter; similar responses were recorded from geniculate cells. *A*, unit giving 'on' responses; *B*, unit giving 'off' responses. 1, cat awake; 2, cat asleep. Upper line in each record indicates when light is on. Second beam in *A* is swept from below upwards, sweeps triggered by spikes; positive deflexions are to the right. Third beam, continuous record from micro-electrode, positive downward. Fourth beam, surface electrocorticogram. Second and fourth beams omitted from *B*. Time, 1 sec.

No attempt was made to compare quantitatively the responses to light stimuli in waking and sleeping cats, since the amount of light reaching the retina was not necessarily the same in the two states. This was not thought to be important for the qualitative observations on firing patterns, since these held over the entire available range of stimulus intensities (2–3 log. units).

Responses of geniculate units to restricted light stimulation

By exploring the visual fields of the cat with a small spot of light one could sometimes find a restricted region over which a geniculate unit could be activated. It was only rarely that a receptive field could be thoroughly explored, since a waking cat seldom kept its eyes fixed for more than a few

minutes. The unit of Text-fig. 5 was recorded from a cell in the lateral geniculate. A 2° spot of light, positioned on the screen for maximal response, gave strong activation during the period of illumination. An annular stimulus centred over the same region gave almost complete cessation of firing, with an 'off' discharge (Text-fig. 5*B*). The retinal area over which this unit could be influenced (the receptive field) thus consisted of a central region from which activation was produced, and a peripheral inhibitory ('off') region.



Text-fig. 5. Responses of a geniculate 'on-centre' cell to restricted (*A*, *B* and *C*) and diffuse (*D*) light stimuli. *A*, 2° spot positioned on screen for maximum response. *B*, annular stimulus concentric with position occupied by previous circular spot. *C*, responses to 2° spot moved horizontally back and forth across receptive field; downward deflexion of upper beam indicates movement of spot to the right. *D*, cat asleep; diffuse light stimulation. Positive downward; time, 1 sec.

Each unit responded to illumination of a restricted portion of the visual field. In the six units studied satisfactorily with 2° spots it was clear that receptive field arrangements were qualitatively similar to those of retinal ganglion cells (Kuffler, 1953), that is, they had an 'on' centre and an 'off' periphery, or an 'off' centre and an 'on' periphery.

Moving a spot horizontally back and forth across the receptive field of the unit of Text-fig. 5 evoked, at each crossing, responses of about the same magnitude for the two directions (Text-fig. 5*C*). This equality of response to the two directions of horizontal movement was a constant finding in geniculate units, and was in marked contrast with the pronounced differences seen in many cortical cells.

The lower record of Text-fig. 5 shows responses of the same unit to a diffuse light stimulus, with the cat asleep. Clusters were present, and, in typical fashion, were most prominent during periods when the unit was least activated—in this unit, preceding and following the stimulus.

'On'-centre units gave 'on' responses to diffuse light, as is illustrated in Text-fig. 5*D*. Similarly the 'off'-centre units studied gave 'off' responses to diffuse light. Thus in both types of unit the central type of response prevailed over the peripheral. Units giving 'on-off' responses to diffuse light were not common, and in this series none were examined with restricted light stimulation.

Optic tract recordings

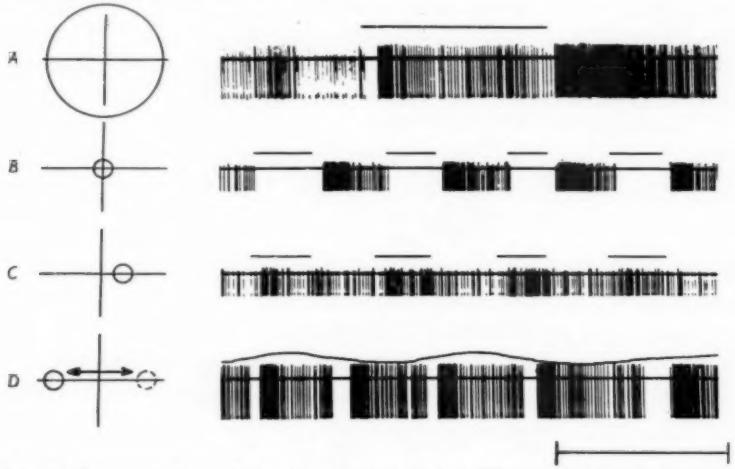
Fibres from retinal ganglion cells were studied in the optic tract. As with geniculate units, the location of the electrode tip was established histologically. In one experiment, for example, electrolytic lesions were made while recording from two successive units. A frontal section through the two lesions is shown in Plate 3, and leaves no doubt that the structure giving these records was optic tract.

As the electrode advanced through the optic tract many units were observed in succession, all fulfilling criteria for fibre spikes. Responses to diffuse light stimuli were very brisk. In contrast with records from geniculate units, clustered firing patterns were never seen. These features, a succession of fibre records in the absence of cell records, brisk responses to light, and an absence of clusters, became the criteria by which optic tract records were, in practice, recognized.

A set of records from an optic tract unit is shown in Text-fig. 6. In the diagram to the left of each record the centre of the receptive field is indicated by the point of intersection of the two axes. In this unit diffuse light evoked responses following both 'on' and 'off' (Text-fig. 6*A*), the 'on' component being preceded by a brief period of inhibition. A 2° spot positioned for maximum response completely suppressed firing, and gave 'off' discharges (*B*). When the spot was moved 2° to either side, 'on' responses were evoked (*C*). These results are in agreement with those of Kuffler (1953), who used the term 'off-centre' to designate this type of receptive field. Each time the spot was moved across the receptive field (Text-fig. 6*D*) firing was interrupted briefly and a brisk discharge followed. As with geniculate units, the size of the response was independent of the direction of horizontal movement.

Responses of an 'on' centre unit are shown in Text-fig. 7. In this example diffuse light and a 2° spot both evoked 'on' responses. Horizontal spot movement across the receptive field produced a discharge at each crossing, followed by a pause in firing. The sequence was thus the reverse of the previous example. Responses to the two directions of horizontal movement were equally brisk. The periphery of this receptive field was apparently not very effective, since

no 'off' response could be evoked with a 2° spot, and since the response to diffuse light contained no 'off' component.



Text-fig. 6. Responses of an optic tract 'off-centre' unit.' *A*, diffuse retinal illumination, evoking a mixed response. *B*, central-type 'off' responses evoked by a 2° spot of light. *C*, peripheral-type 'on' responses to the same spot, moved 2° to the right. *D*, 2° spot moved horizontally back and forth across the receptive field; downward deflexion of upper beam indicates movement of spot to right. Lower beam, positive deflexions downwards. Time, 1 sec.

Text-fig. 7. Optic tract fibre ('on-centre'). *A*, response to diffuse light stimulation; *B*, responses to a restricted light spot, at first moving, then stationary. Upper beam is blanked when light is off; downward displacement indicates movement of spot to right. Lower beam, positive deflexions downwards. Time, 1 sec.

DISCUSSION

In contrasting the present findings with previous unit studies in the cortex, one may consider separately responses to diffuse light; patterns of firing and arousal effects on these patterns; and responses to restricted spots of light, both stationary and moving. The marked differences, especially between geniculate and cortical units, suggest that the cortex has complex integrative functions.

Diffuse light

One of the most striking differences between geniculate and cortical cells was to be found in the degree to which they responded to diffuse light stimulation. Previous studies (Hubel, 1959; Hubel & Wiesel, 1959) showed that most cortical cells are activated poorly or not at all by diffuse stimulation of large retinal areas. This relative ineffectiveness of diffuse light may be compared with the brisk activation of the same units in response to restricted light stimuli, and also with the effectiveness of diffuse light in activating geniculate and optic tract units. It has usually been possible to identify cortical units responding briskly to diffuse light as fibres, and those shown histologically to be in grey matter have so far all been in the deeper layers (V and VI) where specific afferent fibres are known to be plentiful. Because these units had the same firing patterns and responses to diffuse and restricted light as were found in geniculate cells, it is likely that they were the axons of these cells.

It is concluded that most of the cortical units described in previous studies as responding vigorously to changes in diffuse illumination (Hubel, 1957*a*; 1958*a*, *b*) were afferent fibres. In recordings from visual cortex in the 'encéphale isolé' cat, Jung and his co-workers (Jung, 1953, 1958; Jung & Baumgartner, 1955) distinguished units unresponsive to diffuse light (A units) from those responding with 'on', 'off', or 'on-off' discharges (B, D and E units). It is likely from the present findings that any afferent fibres included in the series by these authors would have been classed as B, D or E units. Some units so classed were probably cells, since some cortical cells do respond to diffuse light, although weakly in comparison with their responses to restricted light stimuli. It is probable that 'A units' belonged to the large group of cortical cells which can be activated by restricted light stimuli, but not by diffuse light.

Patterns of firing

In geniculate units there was a strong tendency for discharges to occur in repetitive bursts or clusters. Such clusters were not seen in optic tract fibres. Though grouped firing was common in the cortex (Hubel, 1959) the bursts were longer, less regular, and of lower frequency. Firing in short, high-frequency bursts has been observed in many parts of the nervous system, in maintained activity and in response to natural or electrical stimulation (for reviews, see Rose & Mountcastle, 1954; McIntyre, Mark & Steiner, 1956). Maintained firing of this type was observed by Adrian & Moruzzi (1939) in neurones of the pyramidal tract, groups of two or three closely spaced impulses being synchronized with individual surface slow waves of the motor cortex. At that time it was not clear whether the high-frequency activity was normal or was related to the use of anaesthetics. The present observations show that clustered firing can occur in the central nervous system as a natural event,

unrelated to local injury (as shown by the disappearance of clusters with arousal and by their occurrence in records from fibres), to anaesthetics, or to artificial stimuli.

In the geniculate, clusters were seen only during natural sleep, and their abolition by arousal was not dependent on changes in the light reaching the retina, since the effect was reproducible in complete darkness. Thus it seems clear that geniculate neurones can be influenced by means other than visual, although from the present study there is nothing to suggest which of the afferent pathways to the geniculate mediates these arousal influences. There is an interesting parallel between effects of arousal on cortical cells (Hubel, 1959) and on geniculate units. In both, grouped firing was smoothed out, and discharges became more or less random. Since cortical cells were not easily activated by visual stimuli when the animal's eyes were closed (the light being diffuse), the influence of specific sensory stimulation on their firing patterns could not be studied in natural sleep.

For clusters to appear in the geniculate, a particular set of circumstances is required. The animal must be drowsy or asleep, and the unit must not be activated by visual means. In the aroused animal a geniculate unit may be made, by light stimuli, to vary in its excitation from very rapid firing to complete cessation, without showing clustered firing at any stage. How the sleeping state influences the cell so that at lower levels of excitation clustering occurs is at present obscure, and may only be clarified by intracellular methods.

Restricted light stimuli

The concentric receptive field organization described for the retinal ganglion cell by Kuffler (1953), and confirmed in the optic tract in the present paper, has here been found to hold for units of the dorsal lateral geniculate. In both geniculate and optic tract it has been established that crossing a receptive field with a small spot of light evokes responses which are not significantly different for different directions of crossing. In a previous paper (Hubel & Wiesel, 1959) it was found that receptive fields in the striate cortex do not generally have a concentric arrangement of excitatory and inhibitory areas, and that responses to moving stimuli usually vary with direction of movement. From present results it seems likely that these complex fields and movement responses are a product of the integrative capacity of the striate cortex, since they are not found in geniculate or retinal units.

SUMMARY

1. A method is described for stereotaxic recording from single units from subcortical structures in the unanaesthetized, freely moving cat.

2. Patterns of firing and responses to diffuse and restricted light stimuli

were studied in optic tract, lateral geniculate body and optic radiations; these were compared with previous findings in cells of the striate cortex.

3. Most geniculate cells responded briskly to diffuse light stimuli. In sleep, they tended to fire impulses in brief, high-frequency clusters. Arousal, or activation by light stimulation, abolished these clusters.

4. Similar firing patterns and responses were recorded from fibres in the optic radiation and the striate cortex below layer IV. These fibres were presumed to be geniculate axons.

5. Receptive fields were studied in a small number of geniculate neurones. These had a concentric arrangement of excitatory and inhibitory ('on' and 'off') regions, similar to that described for retinal ganglion cells by Kuffler (1953). This arrangement was confirmed for units in the optic tract.

6. A spot of light moved across the receptive field of a geniculate or optic tract unit produced responses which were independent of the direction of movement.

7. From a comparison of optic tract and geniculate units with cortical cells, in their responses to diffuse and restricted light stimuli, it is concluded that the cortex is the site of complex integrative processes.

I wish to express my gratitude to Miss Kathleen Frank and to Mr C. O. Henson for their unfailing technical assistance, and to Dr W. J. H. Nauta and Mrs M. H. Albrecht for preparing the histological material.

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EXPLANATION OF PLATES

Plate 1

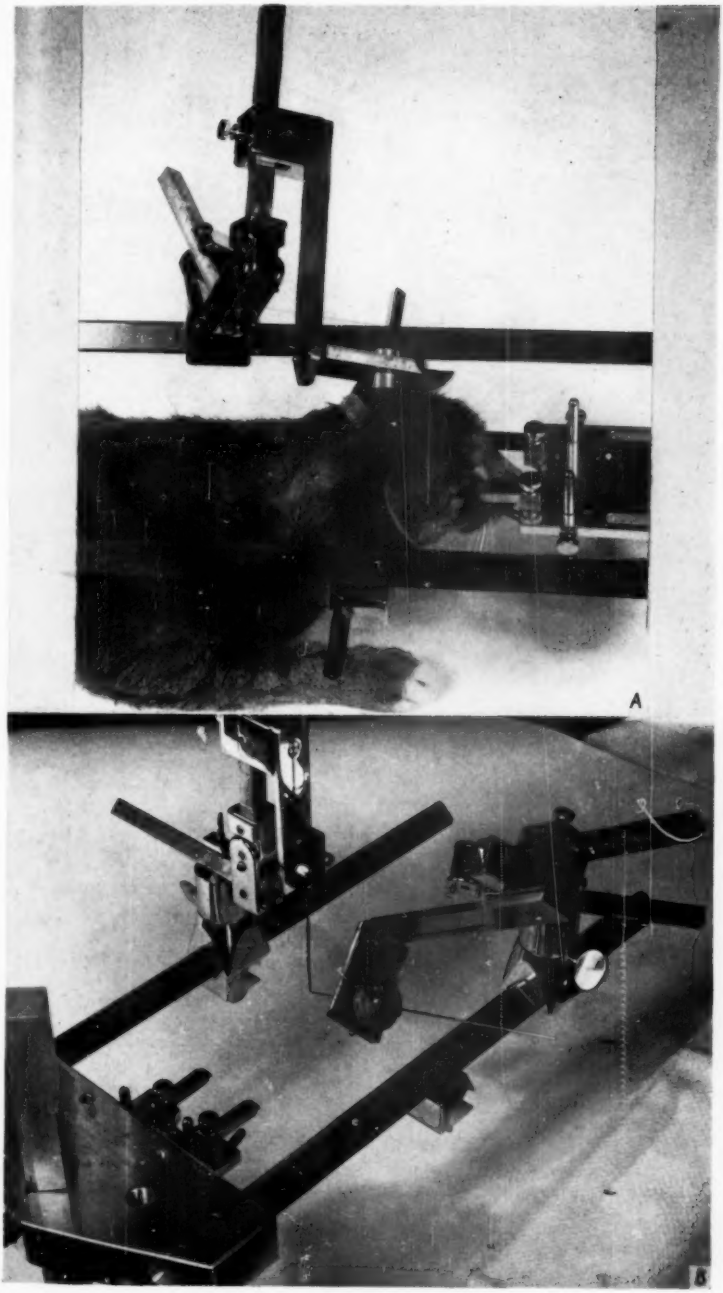
Procedure for adjusting adapter. (*A*) Anaesthetized cat is in Horsley–Clarke instrument. An implant for cortical recording has been placed over the right striate cortex, and a depth-recording implant over the left hemisphere, further laterally. The brass substitute peg, held by an electrode carrier, covers the depth-recording implant: its lower part and a portion of its upper are visible. After the cat is removed, advancing the brass peg along its own axis brings it into the position previously occupied by the implant. (*B*) Adapter is supported on the substitute peg, which has now been advanced to take the position previously occupied by the implant. Target is indicated by vertical pointer held in the electrode carrier to the left of the photograph. A 19-gauge stilette is put through the director hole, and the adapter is adjusted so that the stilette's tip touches the tip of the vertical pointer.

Plate 2

Coronal section (paraffin, cresyl violet stain) through right lateral geniculate body, showing electrode track and three electrolytic lesions made during a penetration (indicated by arrows). Peg was implanted over left hemisphere; track proceeds from left to right and ventrally. *A*, low power, scale 1 cm; *B*, high power, scale 2 mm.

Plate 3

Coronal section through left optic tract, showing two electrolytic lesions made while recording from two optic tract fibres. Electrode proceeded from right to left and ventrally. *A* low power, scale 1 cm; *B*, high power, scale 2 mm.



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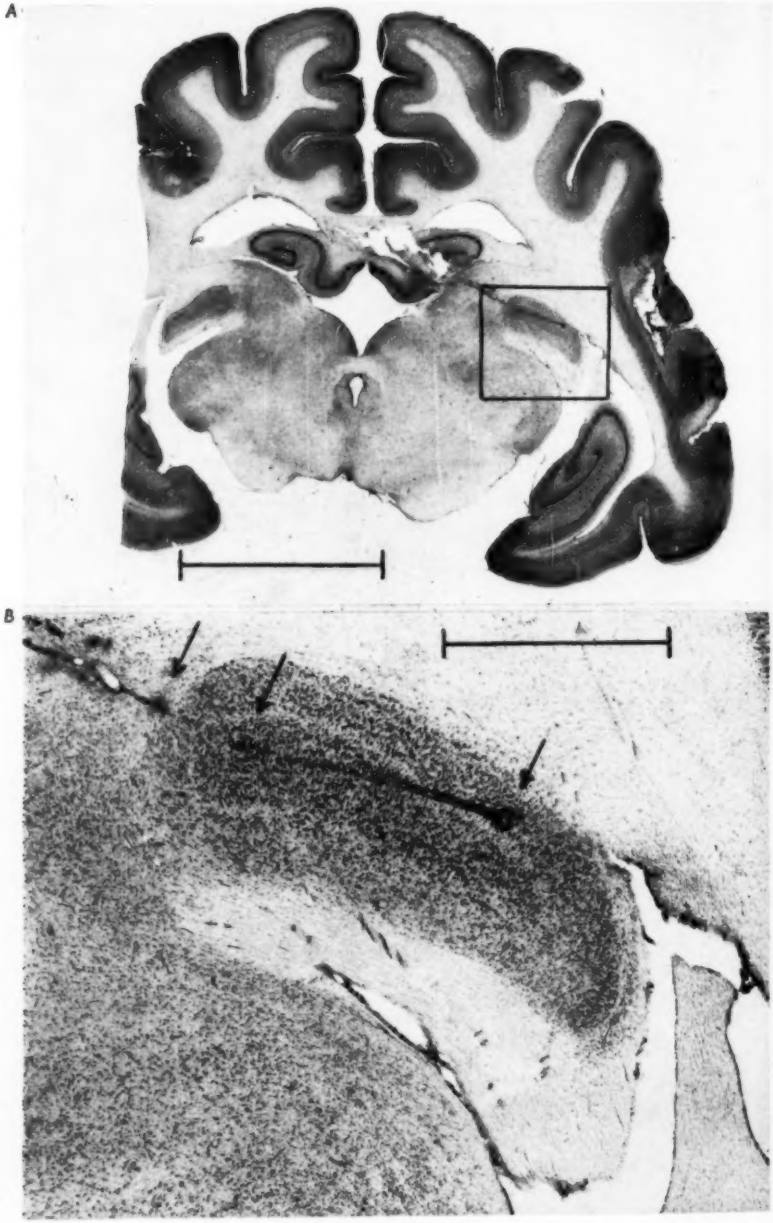
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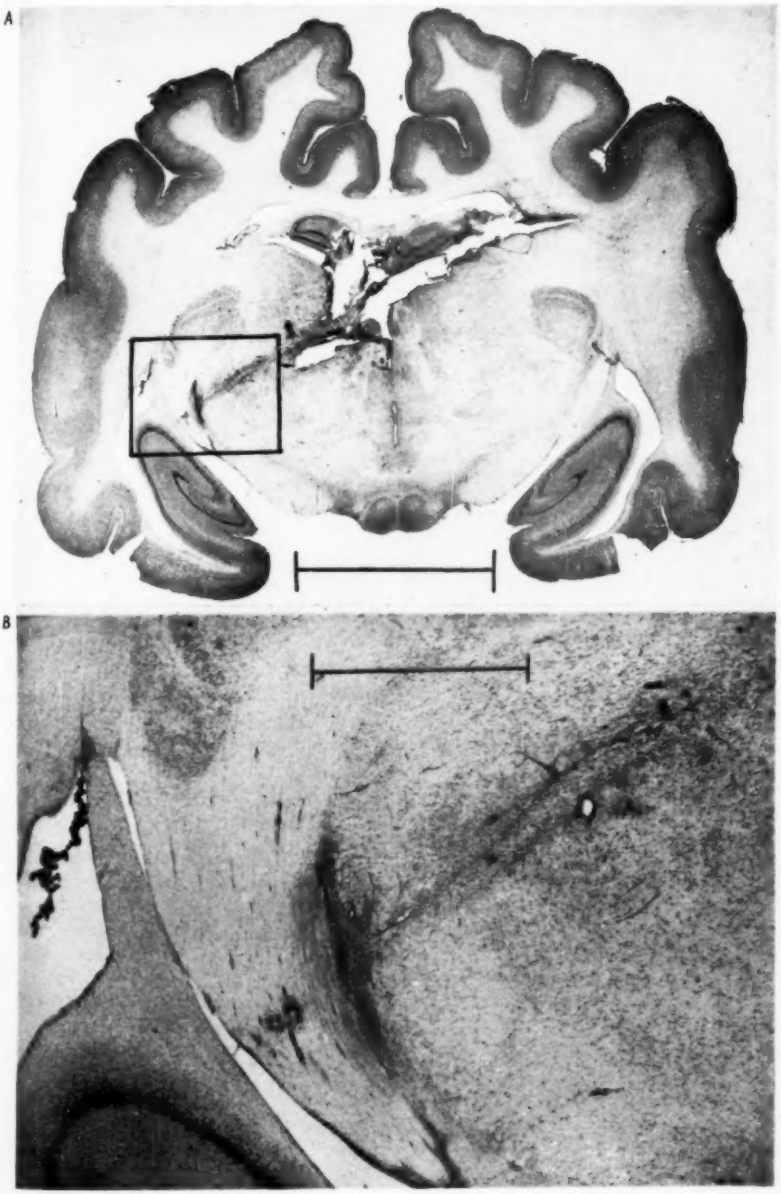
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J. Physiol. (1960), **150**, *pp.* 105–113
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THE EFFECTS OF ELECTRICAL STIMULATION OF VISCERAL AFFERENT NERVE FIBRES ON MONOSYNAPTIC AND POLYSYNAPTIC REFLEX RESPONSES

BY M. H. EVANS AND A. McPHERSON

From the National Institute for Medical Research, Mill Hill, London, N.W. 7

and the Medical Research Council Clinical Research Wing, Institute of Orthopaedics, Stanmore, Middlesex

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Stimulation of visceral afferent nerve fibres evokes activity in skeletal muscles (Miller & Simpson, 1924; Mellanby & Pratt, 1940; Downman & McSwiney, 1946) and leads to alterations in somatic reflexes (Dusser de Barenne & Ward, 1937; Schweitzer & Wright, 1937).

The first investigation of the nature and time course of viscero-somatic interaction was made by Downman (1955) in anaesthetized, decerebrate and spinal cats. Later, Evans & McPherson (1958) showed that electrical stimulation of the afferent fibres in the sympathetic chain or splanchnic nerves facilitated monosynaptic reflexes and inhibited long-latency polysynaptic reflexes elicited from the hind-limb nerves of chloralosed and spinal cats. The present paper describes additional features of these interactions, and the interactions that follow stimulation of the pelvic, hypogastric and vagus nerves.

METHODS

The experiments were carried out on forty cats weighing 1·7–5·9 kg, anaesthetized with intravenous chloralose (70 mg/kg); in a few of these the spinal cord was later transected at the level of C2. Some cats were decerebrated under ether anaesthesia; details of the decerebration procedure have been given in a previous paper (Evans & McPherson, 1959). In order to maintain the cats in good condition all operative procedures were carried out while keeping the cat's rectal temperature between 31 and 33° C. In addition, 5% (w/v) sterile glucose solution was infused intravenously at a rate of about 0·25–1 ml./min throughout the experiment. Before decerebration the cut edge of the skull was plugged with a mixture of bone wax and talc as the bone was removed. The urinary bladder was kept empty throughout all experiments by means of an indwelling catheter.

The hypogastric and pelvic nerves were stimulated by means of conventional indwelling platinum electrodes. Stimulation of other nerves and nerve roots and also recording from them was similar to the technique described previously (Evans & McPherson, 1958).

RESULTS

The interaction between visceral afferent impulses and somatic reflex arcs was studied by observing the effects of stimulation of visceral nerves on mono- and polysynaptic responses elicited from the sixth and seventh lumbar (L6 and L7) and first sacral (S1) segments of the spinal cord. In the case of monosynaptic responses the peak amplitude was measured. Polysynaptic responses whose latency was 2–5 msec have a similar 'spike' form to monosynaptic reflexes and the peak amplitude of these responses was measured.

Effects of stimulation of the splanchnic and pelvic nerves and of the sympathetic chain

Cats anaesthetized with chloralose. When a monosynaptic test response was preceded by a single conditioning stimulus to a splanchnic or pelvic nerve or to the sympathetic chain in the thorax, and the interval between these two stimuli was varied from 0 to 300 msec, it was found that the monosynaptic response was increased when the interval was short and was often decreased when it was longer.

In the case of the splanchnic nerve and sympathetic chain no significant difference was seen in the patterns of interaction of the monosynaptic test response. The shortest interval between the conditioning and test stimuli at which the monosynaptic test response was increased varied from experiment to experiment, ranging from 15 to 25 msec. The longest intervals between conditioning and test stimuli at which the monosynaptic test response was increased, varied from 35 to 60 msec.

In the case of the pelvic nerve the shortest interval between conditioning and test stimuli at which the monosynaptic response was increased varied in different experiments from 23 to 40 msec, and this increase was still present at intervals that varied between 50 and 62 msec. The upper graph in Fig. 1 illustrates an experiment in which monosynaptic test responses, recorded from the left L7 ventral root following stimulation of the left L7 dorsal root, were preceded by conditioning stimuli to the left pelvic nerve. The monosynaptic response was increased when the interval between conditioning and test stimuli was 23–62 msec, reaching a maximum of about 400 % of the control level at an interval of 37 msec. Stimulation of the left pelvic nerve also increased the monosynaptic response elicited from right-sided spinal roots. A decrease in the monosynaptic response after stimulation of the splanchnic or pelvic nerves or sympathetic chain was less frequently produced and was less pronounced than the increase. It occurred when the conditioning stimulus preceded the test stimulus by 50–80 msec. In the experiment illustrated in the upper part of Fig. 2 the

monosynaptic response was recorded from the left L7 ventral root. Following stimulation of the left splanchnic nerve the monosynaptic response was increased in size (to more than 500%) when the interval between the two stimuli was 22–57 msec, but decreased below the control level when the interval between the two stimuli was 65–80 msec. Figure 3 illustrates a rare finding in chloralosed cats; the monosynaptic response was slightly decreased at short intervals, i.e. 20–30 msec.

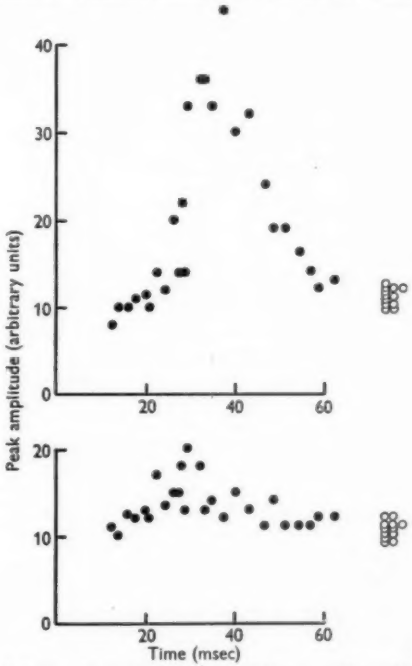

Fig. 1. Graphs of the peak amplitudes, in arbitrary units, of the monosynaptic (top) and early polysynaptic (bottom) responses recorded in the left L7 ventral root following stimulation of the L7 dorsal root (ordinates), plotted against the time between the conditioning stimulus to the left pelvic nerve and the dorsal root stimulus (abscissae). Early polysynaptic latency approx. 1 msec longer than the monosynaptic latency. Cat anaesthetized with chloralose; ○, unconditioned responses; ●, conditioned responses.

When polysynaptic responses were examined it was found that the effects on their peak amplitudes of conditioning stimuli to the splanchnic and pelvic nerves or to the sympathetic chain depended on the latency of these polysynaptic responses. A polysynaptic response whose latency was approximately 1–3 msec longer than the accompanying monosynaptic response was increased in amplitude at intervals which were similar to

those at which the monosynaptic response was increased. As the interval between the conditioning and test stimuli was increased, the early polysynaptic response increased at slightly shorter intervals than the monosynaptic response. These findings are illustrated in the lower graphs of Figs. 1, 2 and 3.

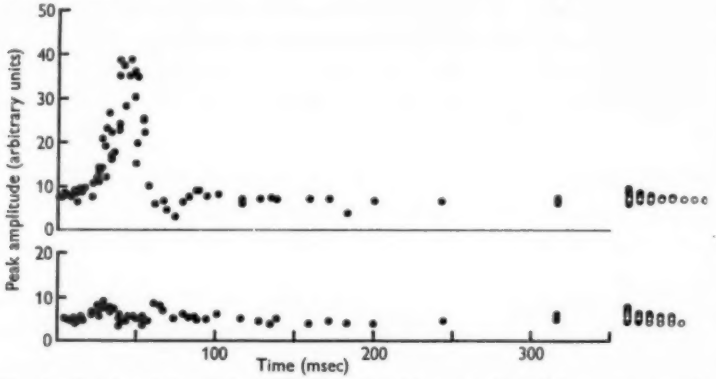

Fig. 2. Graphs of the peak amplitudes, in arbitrary units, of the monosynaptic (top) and early polysynaptic (bottom) responses recorded in the left L 7 ventral root following stimulation of the L 7 dorsal root (ordinates), plotted against the time between the conditioning stimulus to the left splanchnic nerve and the dorsal root stimulus (abscissae). Monosynaptic latency 1·3 msec; early polysynaptic latency 2·4 msec. Cat anaesthetized with chloralose; symbols as in Fig. 1.

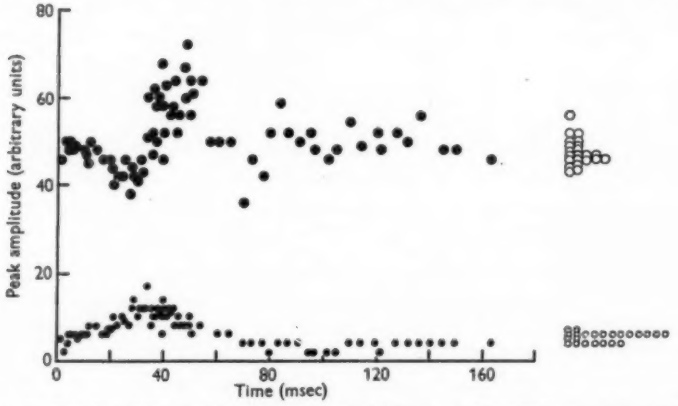

Fig. 3. Graph of the peak amplitudes, in arbitrary units, of the monosynaptic (top) and early polysynaptic (bottom) responses recorded in the right L 7 ventral root following stimulation of the right posterior tibial nerve (ordinate), plotted against time between the conditioning stimulus to the left pelvic nerve and the tibial nerve stimulus (abscissa). Monosynaptic latency 2·8 msec; early polysynaptic latency 4·9 msec; cat anaesthetized with chloralose; symbols as in Fig. 1.

The more complex polysynaptic responses, whose latency was more than 10 msec longer than that of the accompanying monosynaptic response, were not accurately measured but were never apparently increased in area by conditioning stimuli and were almost invariably decreased at intervals between about 20 and 200 msec, often to the point of extinction.

Decerebrate cats. The effects on monosynaptic test responses of a preceding conditioning stimulus to a splanchnic or pelvic nerve or to a sympathetic chain were essentially similar to those in chloralosed cats. In decerebrate cats, however, the increase in amplitude of the responses was less pronounced than that in chloralosed cats; it started at longer intervals

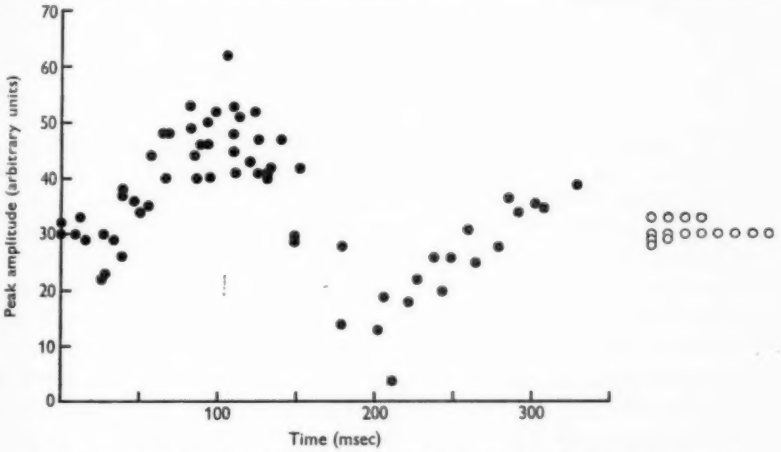

Fig. 4. Graph of the peak amplitudes, in arbitrary units, of the monosynaptic responses recorded in the left L6 ventral root following stimulation of the L6 dorsal root (ordinate), plotted against the time between the conditioning stimulus to the left splanchnic nerve and the dorsal root stimulus (abscissa). Decerebrate cat; symbols as in Fig. 1.

between the two stimuli, varying between 19 and 40 msec; and it continued to longer intervals between the two stimuli (35–150 msec). Figure 4 illustrates such an experiment, in which monosynaptic test responses were recorded from the left L6 ventral root following stimulation of the left L6 dorsal root. The conditioning stimulus was applied to the left splanchnic nerve. The monosynaptic response was increased when the conditioning stimulus to the splanchnic nerve preceded it by 40–150 msec, reaching a maximum of about 200 % of the control level at 105 msec. In some experiments on decerebrate cats there was hardly any increase in the monosynaptic test response following a conditioning stimulus to a visceral afferent nerve.

In decerebrate cats a conditioned decrease in the monosynaptic test response was also seen and was usually more pronounced than the conditioned decrease seen in chloralosed cats. In the experiment illustrated in Fig. 4 the monosynaptic response was markedly decreased when the conditioning stimulus to the splanchnic nerve preceded it by 180–265 msec, at one interval being reduced to approximately 10% of the test level.

In decerebrate cats both early and late polysynaptic responses were affected by conditioning stimuli to the splanchnic and pelvic nerves and sympathetic chain in the same way as in chloralosed cats.

Spinal cats. In spinal cats the monosynaptic test responses were conditioned in a similar manner to chloralosed cats.

Effects of stimulation of the hypogastric and vagus nerves

In the majority of experiments, both in chloralosed and decerebrate cats, stimulation of the central cut end of either vagus nerve in the neck had no effect on mono- or polysynaptic responses elicited from the lumbar segments of the spinal cord. In one experiment, on a chloralosed cat, strong stimulation (20 V, pulse duration 1 msec) of the right vagus nerve increased the contralateral monosynaptic response when the vagal stimulus preceded it by 65–75 msec. In another experiment, on a decerebrate cat, strong stimulation (70 V, pulse duration 2 msec) of the left vagus nerve slightly increased the ipsilateral monosynaptic response when the vagal stimulus preceded it by about 70–160 msec. Schweitzer & Wright (1937) found that strong stimulation of the vagus nerve inhibited the knee jerk. In the present series of experiments an attempt was made to reproduce the stimulation parameters used by them; the vagus nerve was stimulated repetitively (100 c/s) for periods of 2–20 sec. Such stimuli transiently decreased the ipsilateral monosynaptic responses to a slight degree. This decrease might have been due to a change in the arterial blood pressure, which was not measured.

The conditioning effects of stimulation of the central cut end of a hypogastric nerve were only studied in chloralosed cats, and usually this stimulation had no effect on mono- or polysynaptic responses. In two experiments the monosynaptic responses from the lumbar segments of the spinal cord were increased when the intervals between conditioning and test stimuli were 20–70 msec in one and 50–150 msec in the other. Strong stimuli to the hypogastric nerve had to be used to produce these effects. Figure 5 illustrates the experiment in which the increase occurred when the interval between the two stimuli was between 20 and 70 msec. In this experiment there was also some decrease in the monosynaptic response at

intervals of 100–160 msec and a long-latency polysynaptic response (not illustrated in the figure) was completely depressed at intervals between 20 and 160 msec.

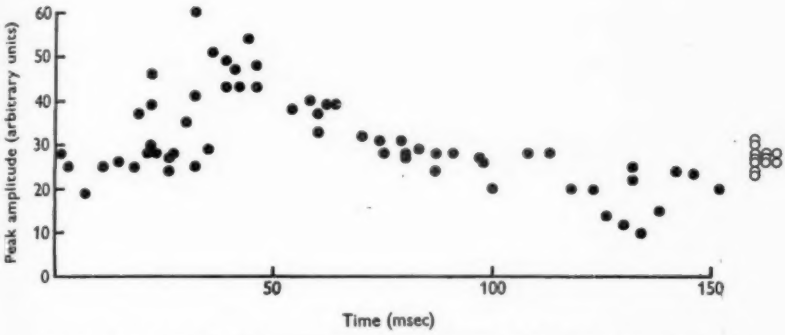

Fig. 5. Graph of the peak amplitudes, in arbitrary units, of the monosynaptic responses recorded in the left L 7 ventral root following stimulation of the nerve to the soleus muscle (ordinate), plotted against the time between the conditioning stimulus to the right hypogastric nerve and the soleus nerve stimulus (abscissa). Cat anaesthetized with chloralose; symbols as in Fig. 1.

DISCUSSION

The experiments described in this paper show that as well as the facilitation of monosynaptic reflexes that follows stimulation of a splanchnic nerve or sympathetic chain (Evans & McPherson, 1958) there is also a period of inhibition of the somatic reflex arcs. In addition, visceral afferent fibres in the pelvic nerves were also shown to be capable of facilitating and inhibiting somatic reflex arcs. Visceral afferent nerve fibres which accompany the vagus and pelvic nerves showed no major differences in their effects on somatic reflexes from those of visceral afferent nerve fibres accompanying the splanchnic nerve and sympathetic chain. The interval between conditioning and test stimuli at which the somatic reflex arc was facilitated was longer in the case of the pelvic nerve than in the case of the splanchnic nerve or sympathetic chain, although the afferent fibres of the pelvic nerve enter the spinal cord nearer to its lumbar segments than do the afferents from the splanchnic nerve or sympathetic chain. This difference in time course could be explained by assuming that the intraspinal pathways of afferent fibres from the pelvic nerve have a greater number of interneurones. The finding that stimulation of the afferent fibres of the vagus nerve in the neck seldom had any effect on lumbar somatic reflex arcs seems to be at variance with that of Schweitzer & Wright (1937) but the latter stimulated the vagus nerve repetitively and examined the

somatic reflexes by studying the knee jerk with intact somatic afferent and efferent pathways.

The hypogastric nerve, which contains few myelinated afferents (Langley & Anderson, 1894–5) also had little effect on lumbar somatic reflex arcs. If, as Amassian (1951) suggests, the effects on somatic motoneurones of stimulation of visceral afferents are mediated by the A gamma-delta group of fibres in the visceral nerves, then stimulation of the hypogastric nerve would not be expected to affect somatic reflexes to any great extent.

Visceral afferent stimuli facilitated monosynaptic responses at certain intervals between the conditioning and test stimuli and this facilitation was followed by inhibition as the intervals were increased. A simple explanation of this would be to assume that visceral afferents can affect motoneurones through an excitatory pathway and also through a more slowly conducting inhibitory pathway. Two such pathways could account for all the commonly observed effects of visceral afferent stimulation on the monosynaptic and early polysynaptic responses. They could also account for the fact that the late polysynaptic response is inhibited at stimulus intervals at which the monosynaptic response is facilitated, because the greater conduction time over the long-latency somatic arcs would lead to the late polysynaptic response falling within the inhibitory phase of the visceral conditioning.

However, the concept of only two types of visceral afferent pathway acting on the motoneurones, one excitatory and the other a slower and inhibitory one, is not entirely adequate, because it does not explain the inhibition of monosynaptic responses sometimes seen at intervals between conditioning and test stimuli of only 20–30 msec. It would seem that visceral afferent impulses can produce additional inhibition of mono- and polysynaptic somatic reflexes, but the mechanisms involved are at present unknown.

The view previously expressed (Evans & McPherson, 1959) that some of the inhibitory effects of visceral distension could be due to bulbar inhibitory mechanisms is supported by the present experiments on decerebrate cats, because pronounced inhibition of mono- and polysynaptic responses could be produced. However, inhibition of mono- and polysynaptic responses was also shown to occur in the spinal animal, and although bulbar influences may be responsible for some of the inhibitory effects of visceral afferent impulses, some inhibition must therefore result from purely spinal mechanisms.

SUMMARY

1. Monosynaptic and polysynaptic responses were used to test the excitability of somatic spinal reflex arcs in the lumbar and upper sacral segments, in cats anaesthetized with chloralose, and in decerebrate or spinal cats.

2. Single-shock stimulation of visceral afferent nerve fibres both facilitated and inhibited monosynaptic reflex arcs. Facilitation usually occurred at intervals between conditioning and test stimuli which were shorter than those producing inhibition. Short-latency polysynaptic arcs were also facilitated but long-latency polysynaptic reflex arcs were inhibited.

3. These excitability changes were commonly seen on stimulation of the afferent fibres in the splanchnic and pelvic nerves and in the sympathetic chain, but were seldom seen after stimulation of the vagus or hypogastric nerves.

We should like to acknowledge the advice and facilities placed at our disposal by Dr W. Feldberg, Sir Charles Harington and Mr H. J. Seddon and also the technical assistance of Mr G. Pierson.

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PREGANGLIONIC SYMPATHETIC ACTIVITY IN NORMAL AND IN RESERPINE-TREATED CATS

By A. IGGO and MARTHE VOGT
From the Departments of Physiology and Pharmacology, University of Edinburgh

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Reserpine causes a loss of noradrenaline and of 5-hydroxytryptamine from the brain, and many attempts have been made to correlate the clinical effects of reserpine with this loss. One of the difficulties in attempting such a correlation lies in the lack of knowledge about the part played by noradrenaline and 5-hydroxytryptamine in normal brain function. Regions containing the central representation of the sympathetic system (hypothalamus, reticular formation of mid-brain and of medulla) are rich in both these amines. If the stores of the amines have an essential role in central sympathetic activity, one might expect such activity to be impaired after the administration of reserpine. The following observations would appear to support such a possibility: peripheral adrenergic neurones fail to excite the tissues they innervate when reserpine has reduced their stores of amines to 10 % or less (Muscholl & Vogt, 1958); continued sympathetic activity, elicited by drugs which stimulate the sympathetic centres, is accompanied by a reduction in the noradrenaline content of these centres, a fact which demonstrates some connexion between nervous activity and noradrenaline turnover (Vogt, 1954).

The object of this work, on which a preliminary note has been published (Iggo & Vogt, 1959), was to test central sympathetic activity in cats in which reserpine administration had reduced the cerebral noradrenaline and 5-hydroxytryptamine to very low figures. Efferent discharge of the preganglionic fibres of the cervical sympathetic was used as a measure of central sympathetic activity. In order to assess possible changes produced by reserpine, it was necessary first to observe the preganglionic sympathetic activity of normal cats in the same experimental conditions, and to devise some simple means of eliciting reproducible changes in activity.

In most of the earlier work (Adrian, Bronk & Phillips, 1932; Bronk, Ferguson, Margaria & Solandt, 1936; Bronk, Pitts & Larrabee, 1940), which has provided the fundamental information on sympathetic discharge, records were derived from whole nerves, whereas in the

present work recordings were obtained from small nerve strands. It was therefore necessary to establish a normal base line for this type of recording.

METHODS

Cats were used for all the experiments. They were anaesthetized with chloralose (80 mg/kg I.V. in 0·9 % NaCl solution) after induction with ether. The reserpine-treated animals had to be induced very slowly with a minimal amount of ether so as to avoid the danger of cardiac failure, and the chloralose was also given very slowly. As a result, some of the cats were only lightly anaesthetized after receiving the full dose of chloralose. If the anaesthesia did not deepen sufficiently within 30 min, a supplementary dose of chloralose was given. During the experiments, which lasted 4–11 hr, further injections of chloralose were given as required in order to prevent the appearance of jerks in response to sensory stimuli.

Intravenous injections. The drugs were injected into a cannula introduced into a femoral vein and connected to a burette.

Recording methods. Electrical activity in the sympathetic fibres was recorded in the mid-cervical region. The right cervical sympathetic nerve was exposed by making a mid-line incision through the skin and separating the neck muscles. The nerve was dissected to free it from the carotid artery but was usually left attached to vagus. A black Perspex plate was placed underneath the nerve, and the dissection of fine strands from the nerve was done on the plate. A trough formed by tying the edges of the skin wound to a metal ring fixed above the neck was filled with paraffin, so that the sympathetic nerve was completely immersed in the paraffin.

The fine strands were dissected from the sympathetic nerve with the techniques and precautions which have been described (Iggo, 1957). No special attempt was made to isolate single units. Electrical activity was recorded by placing the central ends of the fine strands which had been cut distally across a pair of Ag–AgCl wire electrodes. The potentials picked up by the electrodes were amplified with a condenser-coupled amplifier, displayed on a cathode-ray tube and also fed into an audio amplifier. The amplified action potentials, blood pressure signal, respiratory signal and acoustic signal were simultaneously recorded photographically on the same piece of moving bromide paper. Most of the figures in this article are from such photographs. The electrocardiogram was recorded in the initial experiments through leads inserted under the skin of the right foreleg and the left hind leg.

Blood pressure. The cats were given heparin after all the necessary dissection was finished. The left femoral artery was cannulated and connected to a mercury manometer so as to give a continuous visual check on the arterial pressure. A lumbar puncture needle was introduced into the right femoral artery, and the needle was connected to an electromanometer, based on a design published by Green (1954), and used for photographic records of the pressure. This manometer had a frequency response of 35/sec with the long cannula used, and so reproduced faithfully the pressure changes in the artery.

Respiration. Changes in the intratracheal pressure were recorded by inserting a hollow needle, connected to a condenser manometer, into the rubber connexion of the tracheal cannula. The manometer was not sufficiently sensitive to record changes of pressure when the tracheal cannula was open and the cat was breathing naturally, but gave satisfactory records of respiratory efforts during asphyxia with the tracheal cannula closed, or when the cat was artificially ventilated by a pump. Visual observations were made of the relation between respiration and blood pressure when the cat was breathing naturally.

Acoustic stimuli. Clicks were produced by using a large switch, which was opened and shut when required. A signal of the clicks was obtained by putting the switch in a circuit which fed a current to the Y-plates of the cathode-ray tube. Thus when the switch was opened or closed a condenser discharge was recorded.

Reserpine treatment. Reserpine (Serpasil; Ciba Ltd.), 1 mg/kg, was injected intraperitone-

ally into cats on four consecutive days, and the experiment was carried out on the fifth day. There was always loss of weight, and occasionally one of the doses was reduced to half when the cat appeared to be in a poor condition. All the cats drank a great deal of milk, but had to be roused to take their food. The degree of ataxia and sedation tended to be less on the last day of treatment. Miosis, relaxation of the nictitating membrane and blepharospasm were very evident.

RESULTS

Normal cats

Effects of anaesthetic on the level of activity. Chloralose was used as an anaesthetic in order to avoid the depressing effect of barbiturates. As a result, however, the cats sometimes exhibited jerks in response to sensory stimuli. To prevent any change in the recording conditions which might have resulted from movement of the nerve strand on the recording electrodes, additional anaesthetic was given whenever jerks were present. The level of activity in the efferent fibres was, however, diminished as anaesthesia was deepened. This made the comparison of activity between animals less quantitative and also obscured the long-term changes in activity in any animal in which slowly developing drug actions were followed.

Efferent activity in anaesthetized normal cats. The pattern of efferent activity depended to some extent on the number of fibres in a strand. In order to sample as many preganglionic fibres as possible the strands were not dissected down to single fibres. The dissection was carried to the point of allowing activity in individual fibres to be followed with some certainty. This was done because when long term changes were followed it was necessary to be satisfied that the recording conditions had not changed, and the only satisfactory way to do this was to be able to recognize the impulses in individual fibres. Examples of the general features of the discharge of preganglionic fibres are given in Figs. 1*A* and 8*A*. Impulses appeared in bursts, but there was rarely a silent interval longer than 1 sec unless the animal was deeply anaesthetized. The behaviour of individual fibres was easier to follow when there were only a few active fibres in the strand (see Fig. 2*A*). In the undisturbed cat, the individual cells discharged at low rates, often less than 1/sec, so that frequently one cell discharged only one impulse within each of the bursts seen in the multi-fibre records. If a cell fired several times during a burst of activity the frequency of discharge was rarely higher than 30/sec and there were seldom more than a few impulses at such frequencies. The waves of potential recorded from intact sympathetic nerves by previous workers were probably generated by summed impulses in many, but not all, of the fibres in the nerve, each firing once or at most a few times during one wave.

Several rhythms of activity could be recognized. The most clear-cut was

a *respiratory rhythm* (Fig. 1*A*). Normal respiration does not show on these tracings; since. however, there was always a rise in blood pressure at the end of inspiration, the respiratory rhythm can be identified on the tracings by reference to these pressure rises. Thus Fig. 1*A* shows two such rises in blood pressure during two breaths. The electrical activity during these blood-pressure maxima was always at a minimum. A similar form of electrical discharge was observed by Dontas & Nickerson (1957) in splanchnic nerves. In the cervical sympathetic, the units which became inactive

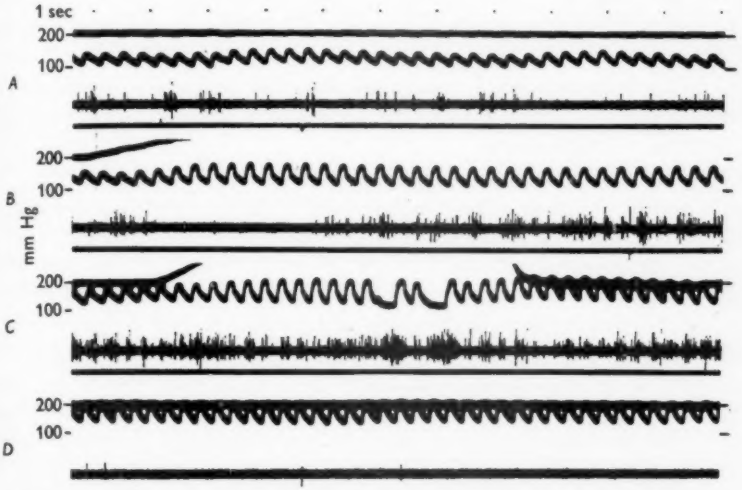

Fig. 1. Normal cat (no. 8). Electrical activity recorded in preganglionic fibres dissected from the cervical sympathetic nerve. Each record shows, from above down, respiration signal (inspiration upwards), arterial pressure (calibration in mm Hg on the left), impulses in the nerve strand, and signal marker for clicks (absent from *D*). *A*, normal activity, with two interposed clicks. The blood pressure rises at the end of each inspiration but, as described in Methods, the recorder for respiration was not sensitive enough to give a deflexion for normal breathing. Records *B* and *C* show the effect of asphyxia; the tracheal cannula was closed at the beginning of *B* and opened again at the end of *C*; two extra-systoles occur in the middle of *C*. *D* immediately after *C*. Time marker, seconds (top of *A*).

during the respiratory rises in blood pressure could also be silenced by pressor doses of adrenaline. Further evidence that the rhythm was secondary to the vascular changes was obtained in records taken during the early phase of, and during recovery from, asphyxia. Thus, for example, in the left of Fig. 1*B*, the abolition of activity coincided with the rise in blood pressure and not with the much more prolonged inspiratory effort. After the trachea was reopened the blood pressure remained elevated and

the efferent activity was suppressed, even though the respiratory activity was enhanced (Fig. 1*D*).

Adrian *et al.* (1932) ascribed the respiratory rhythm they observed in the rabbit to a central interaction between the respiratory and vasomotor centres, whilst Bronk *et al.* (1936, 1940) argued that, in the artificially ventilated cat, it was partly due to an inhibitory effect of the pulmonary stretch receptors. In cats breathing naturally the present work showed a consistent relation between the peaks of the respiratory blood-pressure fluctuations and the inhibition of the efferent discharge. This relation was still evident when the trachea was closed (Fig. 1*B*). In these conditions, however, the pulmonary stretch receptors were not excited during inspiration, and therefore cannot have been responsible for the inhibition of efferent activity. There are obviously a number of mechanisms responsible for respiratory rhythms.

One single unit was isolated in which the discharge of impulses appeared to be directly linked to respiratory activity (Fig. 2). There was a burst of impulses during every inspiration. The discharge of impulses in this unit was not abolished by pressor doses of adrenaline. During asphyxia (Fig. 2*B*, *C*) the discharge was closely linked to the respiratory efforts. This relation can be seen in the figure, in which the end of inspiration is marked by a downward movement of the base line. Thus the first breath after closing the trachea (during the expiratory pause) was longer than normal and the discharge of impulses was also carried on for longer (Fig. 2*B*). As the subsequent breaths became deeper, the efferent discharge was further enhanced. After air was allowed once more into the lungs, the efferent activity reverted to a lower frequency (Fig. 2*D*), and was not abolished, as happened in most other fibres, after a period of asphyxia (see p. 122). The discharge of this exceptional unit thus corresponds closely to the activity described by Adrian *et al.* (1932) in the rabbit, and is quite different from the behaviour to be expected if the efferent discharge had been inhibited by pulmonary stretch receptors, as in the experiments of Bronk *et al.* (1936).

Cardiac rhythm. Although the electrical activity in the undisturbed cat appeared in bursts, there was never any conspicuous relation of these bursts to the heart rate. A definite cardiac rhythm, however, appeared when a high level of efferent discharge was associated with a rise in blood pressure. This combination was found during the later phases of asphyxia, as in Fig. 1*C*. This record also shows details of the relation between the bursts of impulses and the cardiac activity. Two arterial pressure waves are virtually absent in this record, presumably because of extra-systoles. There was a prolonged burst of impulses when the normal pulse was absent. Each burst started 150–200 msec after the preceding peak and

ended shortly after the peak of the next wave. By this fortunate chance it became possible to relate the start of the burst of impulses to the fall of the preceding pulse wave and its end to the pressure wave with which it coincided. Thus the conclusion reached by Adrian *et al.* (1932) and Bronk *et al.* (1936, 1940), that the cardiac rhythm had its origin in a stimulation of the baroreceptors which in turn inhibited the vasomotor centre, is shown to be valid for the individual pulse. One might, therefore, infer that there is a latent period of about 200 msec between stimulation of the baroreceptors and inhibition of the preganglionic sympathetic outflow. That these

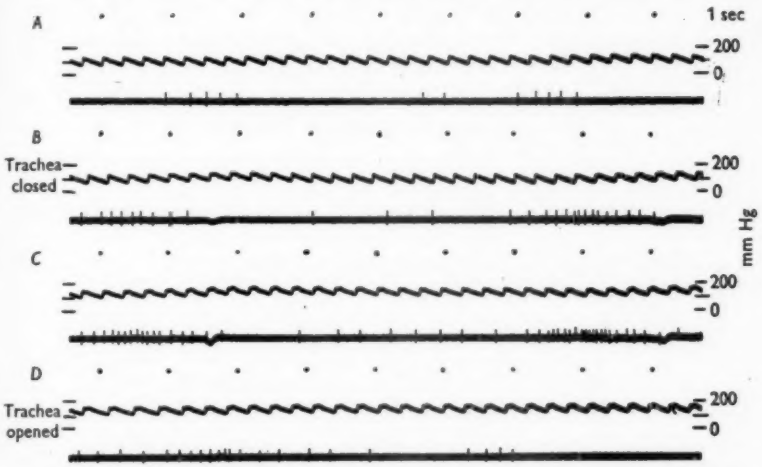

Fig. 2. Normal cat (no. 5). Impulses in a single preganglionic sympathetic fibre: respiratory rhythm. On each record: time (sec), blood pressure (calibration in mm Hg on the right), action potentials. *A*: impulses during two breaths: the peaks of activity coincide with inspiration (not indicated on the record). *B* and *C*: activity during asphyxia. Here the respiratory cycle is recognizable by a short downward movement of the base line which occurred at the end of each inspiration. Trachea closed during the expiratory pause just before *B*; the activity is enhanced during the next breath. 15 sec interval between *B* and *C*; trachea opened just before *D*; the activity quickly returned to the pre-asphyxial pattern; there was no inhibition like that shown in Fig. 1*D*.

bursts of impulses represent vasomotor activity is not certain but is suggested by the fact that they are abolished by a pressor dose of adrenaline.

Bronk *et al.* (1936), in their Fig. 7, also show an example of the presence of cardiac rhythm when the blood pressure was raised, and its disappearance when the pressure was allowed to fall.

A cardiac grouping of the impulses does not necessarily occur when there is an enhanced efferent outflow. For example, there was a massive efferent discharge which showed no cardiac rhythm following an intravenous

injection of a large dose of morphine. This was at a period when the blood pressure was still unchanged, so that one of the conditions in which cardiac rhythm was found, namely high blood pressure, was not satisfied.

Sympathetic activity during Traube waves. The regular waves, known by various names, which appear in most blood pressure records, have been attributed by Guyton & Harris (1951) to 'presso-receptor-autonomic oscillation'. There was one particularly clear example of these Traube waves in the present work. It occurred during a period of asphyxiation in

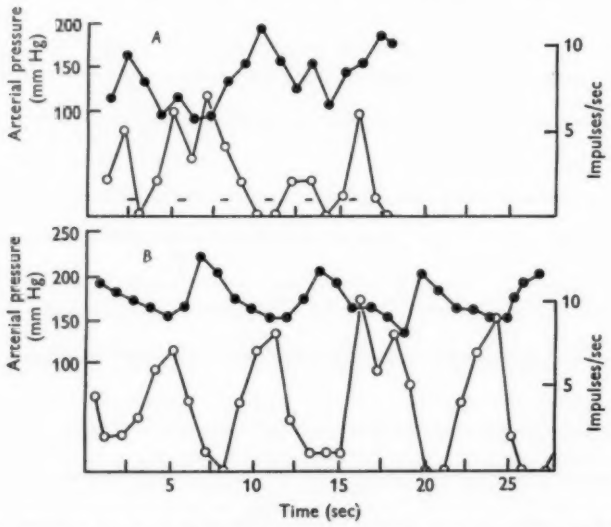

Fig. 3. Normal cat (no. 7). Traube waves in the blood pressure and efferent activity in the cervical sympathetic nerve. The filled circles represent the diastolic blood pressure and the open circles the total efferent activity per second in a multi-fibre strand. In *A* the cat was ventilated by a respiration pump, and the peak of inspiration is indicated by the short horizontal strokes at the bottom of the picture. *B* shows the activity after the respiration pump was turned off; the cat did not attempt to breathe and the Traube waves are very clear.

a cat which had been artificially ventilated. After the pump had been turned off the cat did not attempt to breathe for 1 min, and during this time the Traube waves were present, uncomplicated by respiratory variations in blood pressure. A rhythmical discharge of impulses was also present in the sympathetic fibres (Fig. 3*B*, lower record). It appeared at a gradually increasing frequency as the blood pressure was falling, and then gradually fell off again as the blood pressure rose once more. The period of the Traube rhythm was 6 sec and the efferent outflow lasted 3–4 sec. The blood pressure did not begin to rise until about 2·5 sec after the first

impulses appeared in the strand, and began to fall again about 1 sec after the last impulse in the group. The oscillations can be ascribed to delays in two components of the stabilizing mechanism, (*a*) the central delay along the path, pressoreceptor–central nervous system–preganglionic cell and (*b*) the sluggishness of the blood pressure in responding to changes in the efferent outflow. On the basis of the present results the delay along the central pathway is 200–300 msec and the delay in effector response about 2·5 sec. On this interpretation the Traube waves arise largely because of effector delay.

The Traube wave can be disrupted but not abolished by breathing (Dornhorst, Howard & Leathart, 1952). This is shown in Fig. 3*A*, in which the cat was on artificial respiration (pump strokes marked in the figure). Here complex fluctuations of blood pressure are superimposed on the Traube waves, the electrical record showing equal complexity of rhythm.

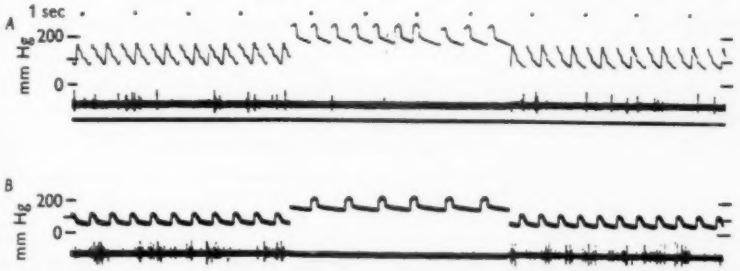

Fig. 4. The effect of intravenous injections of adrenaline on the blood pressure and the efferent discharge in a normal cat (*A*, no. 11), and in a reserpine-treated cat (*B*, no. 6). On the left, the control periods; in the centre, the records 20 sec after an injection of 7 and 5 μg respectively of adrenaline; on the right, records taken 4 min later. Blood pressure calibration (mm Hg) on the left. Time marker, 1 sec (top tracing).

Effect of drug-induced blood pressure changes on efferent activity. As has been previously reported by Adrian *et al.* (1932), most of the activity in the cervical sympathetic nerve can be modified by drugs which alter the blood pressure. When the blood pressure of the chloralosed cat was raised by the intravenous injection of 5–10 μg adrenaline (Fig. 4*A*), the efferent activity became less, and was absent in most of the fibres when the systolic pressure was above 200 mm Hg. At these pressures there was obvious cardiac slowing, the rate falling by as much as one half. Conversely, the efferent activity could be enhanced by lowering the blood pressure with an intravenous injection of 6 μg histamine.

The sensitivity of different fibres to rise in blood pressure varied. Some units were readily silenced, whereas others, e.g. the respiratory unit of

Fig. 2, were resistant. The different units in a strand dropped out at different times as the blood pressure rose, and the activity reappeared in the reverse order.

Effect of asphyxia on the efferent discharge. Temporary closure of the tracheal cannula was used as a convenient way to excite the sympathetic nervous system. The effect in normal cats was influenced by two factors, the depth of anaesthesia and the type of respiration. If the cat was lightly anaesthetized and breathing naturally, the effect was quick in onset. Deeper anaesthesia delayed the rise in efferent outflow. If the lungs were inflated by a respiration pump, there was usually some degree of over-ventilation, since the stroke was adjusted to prevent any respiratory efforts on the part of the animal. As a consequence the onset of the response was later still and initial vigour of changes in efferent activity less.

Asphyxiation of normal cats led after 5–10 sec to a progressive rise in arterial pressure and, as Adrian *et al.* (1932) have reported, to an enhancement of efferent outflow. There was a more persistent and higher frequency of discharge in the fibres already active, and new fibres started firing. At the end of a period varying between 10 and 45 sec the outflow was nearly continuous (Fig. 1*C*). The discharge, however, was not steady; the two patterns of activity described in preceding sections, the cardiac rhythm and the rhythm due to respiratory fluctuations of blood pressure, persisted throughout the enhanced activity. After the air supply was restored by opening the trachea, the efferent activity quickly subsided, often within 5 sec. During this period, the blood pressure remained at the same, or rose to an even higher, level than that reached at the end of the asphyxia. The sudden disappearance of the efferent activity can be accounted for by the fact that the inhibitory effect of the high blood pressure was no longer opposed by the central stimulatory effects of asphyxia.

When normal cats were ventilated with a respiration pump, animals which previously had given a brisk response to asphyxia sometimes did not breathe for a whole minute after the pump had been turned off; the efferent discharge was then also unchanged. Presumably there had been some degree of over-ventilation, although the pump stroke had been so adjusted as to keep the blood pressure at the initial level. This observation illustrates a difficulty in comparing the reactions of the normal and the reserpine-treated cat. All the treated cats had to be ventilated by pump during the experiments to prevent anoxia, so that the possibility of over-ventilation was always present to bedevil the comparison of the two types of preparation.

The one unit which had a primary respiratory rhythm and which was relatively resistant to inhibition by adrenaline also continued its individual pattern during asphyxia (Fig. 2 and p. 118). When the trachea was re-

opened the activity reverted to its original pattern and was not suppressed by the continued high arterial pressure.

The experiments were often terminated by clamping off the trachea. Such 'terminal' asphyxia caused a continuous discharge of impulses in the sympathetic fibres. The discharge became regular about 2–3 min after closure of the trachea, and at different times in different fibres. The steady discharge ranged from 2/sec in some fibres to 25/sec in others. The discharge lasted for up to 9 min and continued after the heart had stopped pumping blood. In all experiments fibres were found which had been silent in the undisturbed animal and which became active in the later stages of asphyxia.

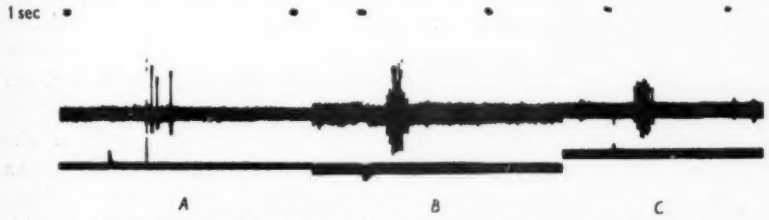

Fig. 5. Normal cats, nos. 7(*A*), 8(*B*), 9(*C*). Response of the sympathetic system to clicks. Each record was selected from a silent part of the base line in order to make the effect clearer. In each instance the click has excited several fibres, and in *B* and *C* there is repetitive firing of individual units. Time marker, 1 sec.

Sympathetic response to acoustic stimuli. Though all cats were anaesthetized sufficiently deeply not to respond to a sudden noise with a jerk, a click usually elicited a burst of impulses. Often new units were excited whilst some of the fibres already firing in the normal bursts appeared unaffected. The time course of the efferent discharge was always the same. Within 100–200 msec of the click there was a burst of impulses lasting 100–300 msec. The individual fibres discharged 1–5 impulses, depending on the excitability of the animal and the intensity of the stimulus. When a fibre fired several times the frequency of discharge could be as high as 100 impulses/sec. Examples are shown in Figs. 1*A*, 8*A* and 5. The response is seen best when there is little other activity (Fig. 5); in Fig. 1*A* the response can be identified by the fact that a new unit which had previously been silent discharged at each of the clicks. Individual units did not respond consistently to repetition of the same stimulus. There appeared to be a relation between the level of activity of a neurone and the likelihood that it would be excited by a click, but the relation was difficult to put on a quantitative basis. Thus a unit with a marked respiratory rhythm did not respond to a click during expiration but did respond during inspiration; later in the experiment, when the anaesthesia had become lighter, the

unit could be excited at any stage of the respiratory cycle. Another unit in the same strand, otherwise silent, fired in response to the click at all phases of the respiratory cycle.

Frequent repetition of the acoustic stimulus led to a failure of the response. After 3 or 4 clicks applied within 10 sec the response was absent, but a short rest of, say, 30 sec was sufficient to restore the response. Electrical stimulation of the saphenous nerve produced a similar response, but was not often used owing to the danger of eliciting reflex movements.

Effect of morphine on the sympathetic system. Morphine was tried as a stimulant for the sympathetic system because it is known to excite the sympathetic centres of the cat (Elliott, 1912). Small doses given intravenously had an uncertain effect on the efferent outflow; some enhancement was apparent but the origin of the effect was in doubt, since morphine is a respiratory depressant and the increased outflow might in part have been secondary to under-ventilation arising from this depression. This was controlled in the later experiments by the use of artificial respiration. When large doses of morphine (3–7 mg/kg) were given intravenously, there was an immediate slight rise in the blood pressure followed by a slowly developing deep fall. The efferent activity was much enhanced within 25 sec, at which time the blood pressure was normal. The activity remained at a high level while the blood pressure was falling and later gradually fell off; within half an hour it was coming in bursts which were associated with the convulsions appearing at that time. In one animal the discharge became very regular, about 10/sec, during the period of enhanced activity, thus giving a most unusual picture. There were various peripheral signs of sympathetic stimulation, such as a dilated pupil and a contracted nictitating membrane.

Reserpine-treated cats

Efferent activity in the undisturbed reserpine-treated cat. Sufficient reserpine was given to cause the disappearance of a large proportion of the catecholamines from the hypothalamus, mid-brain, medulla oblongata and the post-ganglionic sympathetic adrenergic neurones. Actual mean losses of noradrenaline, for two cats in which estimations were carried out after the same treatment, were 90·2 % from the hypothalamus and 94·7 % from the superior cervical ganglia.

Strands of the cervical sympathetic containing active efferent fibres were very readily obtained in these animals. This might indicate that the over-all discharge was greater than normal, but it is impossible to be perfectly certain about it. The activity in individual fibres was more continuous than in normal cats (Fig. 7*A*), even when the blood pressure was above 100 mm Hg; all the animals were ventilated by a respiratory

pump in order to remove any asphyxial drive to the sympathetic nervous system. Some units, in particular, discharged more regularly than had ever been observed in normal cats. Bursts of impulses were present but no respiratory or cardiac rhythm was apparent (Fig. 6).

The blood pressure in the reserpine-treated animals was very steady and exhibited no respiratory rhythm, and we think that for this reason the efferent discharge did not show the fluctuations to be seen in normal

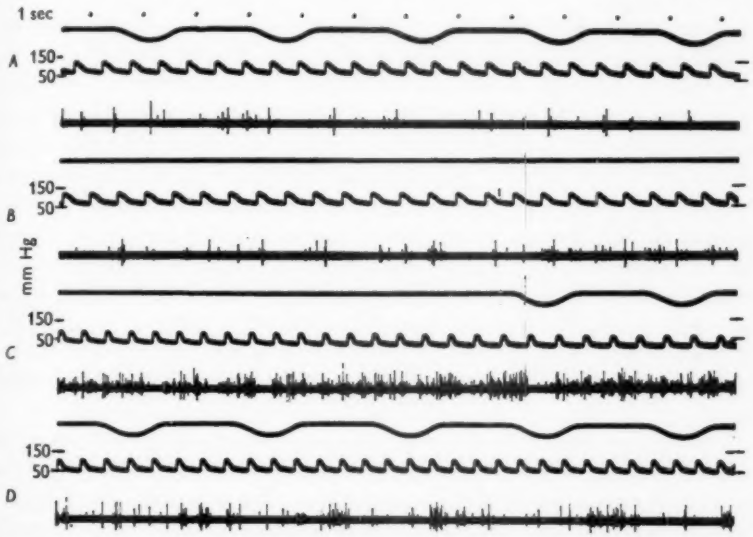

Fig. 6. Reserpine-treated cat (no. 6); effect of asphyxia on efferent sympathetic activity. The cat was artificially ventilated. Top tracing, respiration pump; second tracing, blood pressure (calibration in mm Hg); bottom tracing, efferent discharge. *A*, base line activity. *B* and *C*, activity during asphyxia, interval of 15 sec between *B* and *C*; the pump was turned off at the beginning of *B* and was turned on towards the end of *C*; during asphyxia increase in the efferent discharge but fall in blood pressure. *D*, 2 min after the end of asphyxia; blood pressure not quite back to normal and efferent activity still enhanced.

animals. A cardiac rhythm was seen on some occasions, when the blood pressure was rising after an intravenous injection of adrenaline. There was no cardiac rhythm during asphyxia as there was no rise in blood pressure (see below).

Acoustic stimuli were effective in eliciting bursts of impulses as in the normal animal. The discharge was sometimes more difficult to arouse and was often lost in the high background activity, but was otherwise similar to the response in normal animals; as in the normal preparation not all fibres could be made to respond.

Asphyxia. In reserpine-treated cats, as in the normal animal, closing of the trachea led to an increase in the sympathetic efferent outflow. There were, however, several important differences in the response. The efferent activity was slower in building up. This may have been due to the use of artificial ventilation in the reserpine-treated cats and not to a direct effect of the drug. The ventilation was adjusted to give a stable arterial blood pressure, about 100 mm Hg, but nevertheless the cats might have been over-ventilated.

A second difference from the normal cats was that the enhanced activity persisted after the respiration pump was started again (see Fig. 6). The main reason for this must have been the reversal of the normal blood pressure response to asphyxia. Soon after occlusion of the trachea the blood pressure and heart rate of the reserpine-treated cat started to fall instead of to rise, and on release of the occlusion recovery of the blood pressure was very slow, so that sympathetic activity continued to be stimulated. The absence of the normal rise in pressure and of tachycardia was, of course, caused by the functional failure of the vasoconstrictor nerves, the nervi accelerantes and the adrenal medulla, all of which had lost their stores of catecholamines. That the blood pressure was not even maintained but fell was probably a result of cardiac failure from lack of oxygen in the absence of the tonic action of the sympathetic system on the heart.

A subsidiary reason for the slow return of sympathetic outflow to the resting level might have been that the normal cat increased its respiratory volume by taking larger and faster breaths after a period of asphyxia, so that the normal blood gas levels were quickly restored. In the reserpine-treated animals, the rate and stroke of the pump were not altered so that a longer time would elapse before recovery was complete.

The enhanced sympathetic outflow during the post-asphyxial period failed to elicit any peripheral circulatory response, as is seen by the slowness of the recovery and by the absence of any overswing of blood pressure or pulse rate at any phase of the recovery period (Figs. 6 and 7).

Prolonged asphyxia led, as in normal cats, to a very steady discharge of impulses in individual fibres, at frequencies as high as 25/sec. Terminal asphyxia in the reserpine-treated cat revealed that, at rest, not all the sympathetic fibres had been active so that in this respect also the two preparations were similar. The absence of any peripheral effect of central sympathetic activity did not lead to extreme over-activity of the sympathetic centres.

Adrenaline. The pressor action of adrenaline appeared to be more prolonged than in normal cats. During the pressor effect all the preganglionic sympathetic activity disappeared (Fig. 4*B*). Thus the central mechanisms

for stabilizing the blood pressure were still active, irrespective of the inactivation of some of the effector pathways. Furthermore, an injection of adrenaline, after having produced an initial rise in blood pressure and heart rate, led to a bradycardia as the pressure rose higher still. Thus, wherever the efferent pathways were intact, central control of blood pressure proceeded in the normal way.

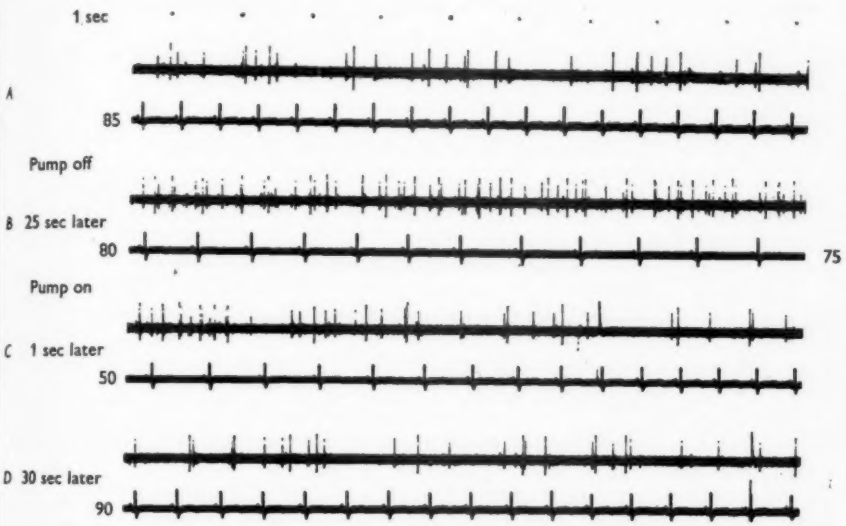

Fig. 7. Reserpine-treated cat (no. 3); effect of asphyxia on efferent sympathetic activity. This preparation shows the regularity of the efferent discharge better than cat 6 (Fig. 6). Top tracing, efferent activity; bottom tracing, electrocardiogram. *A*, base-line activity during artificial respiration; the pump was then turned off for 45 sec. *B*, after 25 sec of asphyxia; increase in the frequency of firing. *C*, immediately after the resumption of artificial respiration. *D*, 30 sec later; heart rate, blood pressure and discharge back to normal. Efferent activity not abolished at any phase of the recovery from asphyxia (contrast normal cat, Fig. 1). Time marker, 1 sec (top tracing); blood pressure (mm Hg) marked at sides.

Morphine. Intravenous injections of morphine in doses of 4–7 mg/kg were given to two cats which had had reserpine previously. In each instance there was a slight increase in efferent activity shortly after giving the morphine, but the effects were less dramatic than in normal animals.

Acute effects of reserpine. Bein (1955) has reported immediate effects of an intravenous injection of reserpine on the electrical activity of a cardiac nerve. He used a cat anaesthetized with chloralose and a dose of 0·1 mg/kg. The electrical activity in the nerve started to decrease 10 min after the injection and had disappeared in 50 min; at this time bradycardia was

apparent. We repeated Bein's experiment in order to see whether a similar decline in activity would occur in the cervical sympathetic nerves.

One difficulty in following changes in efferent activity for an extended period is that the recording conditions may change or that the preparation may deteriorate. When, however, as was done in the present work, the activity is recorded from small strands which permit the identification of impulses in individual fibres, this possibility is much reduced.

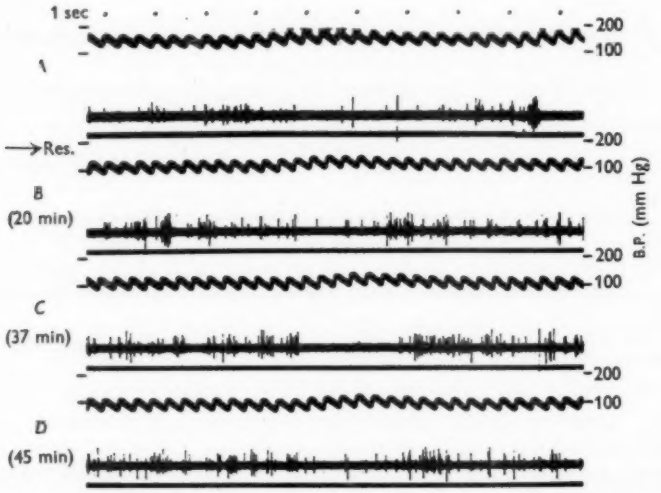

Fig. 8. Normal cat (no. 8); natural respiration. Changes in efferent sympathetic activity following the intravenous injection of reserpine (0·1 mg/kg). Each record shows the blood pressure (calibration in mm Hg on the right), impulses in the nerve strand, and the signal marker for clicks. The records have been aligned so that the rises of blood pressure at the end of each inspiration are in the centre of the picture. *A*, control period, followed immediately by the injection of reserpine (→Res.). *B*, 20 min; *C*, 37 min; *D*, 45 min later. The blood pressure had fallen within 20 min and was lower still at 45 min despite some increase in efferent discharge (*B*, *C* and *D*). No change in heart rate. In all tracings, the efferent activity is lowest during the respiratory rise in blood pressure, but the effect is most pronounced 37 min after reserpine (*C*). Time marker, 1 sec (top tracing).

When reserpine (0·1–0·2 mg/kg) was injected intravenously, a fall in blood pressure developed in the course of about 15 min in those cats in which the initial pressure was high; this dependence of the effect on the initial level has been reported by many authors (see Bein, 1956). Bradycardia was not observed. The effect on the electrical activity in the cervical sympathetic was small and not consistent. Twice a reduced activity was observed 20 min after the injection, but by 50 min the activity was greater than before the drug. In two other cats the only effect was that repre-

sented in Fig. 8, an increased over-all activity with an occasional longer pause between the bursts. These pauses coincided with the rises in pressure which formed part of the normal respiratory fluctuations. It is not possible to exclude some degree of recovery from the anaesthesia as a factor contributing to the enhanced activity.

One experiment was carried out in order to see whether a large intravenous dose of reserpine (1 mg/kg) would produce any effect on the sympathetic activity of a cat previously treated with reserpine (1 mg/kg for 4 days). No such effect was seen.

DISCUSSION

The results in the normal cats in most respects confirm the earlier descriptions of sympathetic activity given by Adrian *et al.* (1932) and Bronk *et al.* (1936, 1940). New information about the response of the sympathetic nervous system to abrupt sensory stimulation is given, in particular the brief and quick discharge of impulses following an acoustic stimulus. The use of fine strands in which the behaviour of individual units could be followed also revealed that, although a cell may not fire continuously at frequencies higher than 25/sec even during the intense stimulation of terminal asphyxia, some fibres at least can respond at much higher frequencies, if only for a short time.

The results on the cats which had been treated with high doses of reserpine were very clear-cut. Spontaneous preganglionic sympathetic activity was unabated, and perhaps enhanced, whilst the treatment had been thorough enough completely to abolish any peripheral sympathetic activity; the latter fact is best illustrated by the effects of asphyxia, which, after reserpine, were hypotension and bradycardia instead of hypertension and tachycardia. Though the experiments gave the impression that efferent preganglionic activity was greater than normal, the technique of recording from small strands of the nerve does not permit any definite statement to be made on the total outflow of sympathetic impulses; it was, however, clear that the discharge was more continuous than, and lacked the fluctuations of, the activity of the normal animal. The obvious explanation is that the lack of any peripheral effects of the central discharge caused a lack of inhibitory feedback which creates various rhythms in the normal resting cat. In both normal and reserpine-treated cats the sympathetic outflow was suppressed when the blood pressure rose, and most effectively by the injection of a pressor drug. That rises in blood pressure could not be produced in the reserpine-treated animal by the usual stimuli to which the normal animal responds with increased vasomotor tone and output of adreno-medullary hormones is, of course, a result of the inactivation of the

post-ganglionic adrenergic neurones and the adrenal medulla, and not of a failure of the centres; neither is it related to impaired transmission in autonomic ganglia, since transmission has been shown to be normal after treatment with reserpine (Trendelenburg & Gravenstein, 1958).

Acoustic stimuli and asphyxia increased the preganglionic electrical activity in the normal and in the reserpinized cat. When, however, air was again admitted to the lungs, the normal cat exhibited electrical silence whereas the reserpine-treated animal showed prolonged enhanced activity. The reason for this change is that, after reserpine treatment, the normal post-asphyxial rise in blood pressure is replaced by a prolonged period of hypotension which acts as a stimulus to the sympathetic centres. The observation that terminal asphyxia causes greatly enhanced discharge also in the reserpine-treated cat shows that, even in the complete absence of peripheral effects, the sympathetic centres do not discharge maximally under resting conditions.

The intravenous injection of morphine appeared to cause a smaller and more evanescent increase of sympathetic activity in the reserpine-treated cats. This may be an example of the central antagonism of reserpine and morphine found on injection of these drugs into the conscious cat (Gaddum & Vogt, 1956), but since dosage and experimental conditions were very different in the present work there might be other interpretations.

The experiments throw no light on the possible role of catecholamines and 5-hydroxytryptamine in central sympathetic activity. To conclude from the results that sympathetic activity of the brain is independent of these amines is not permissible, since these substances continue to be synthesized and never completely disappear from the tissue of the reserpine-treated animal. It is safe, however, to assert that the stores of these amines are not essential for normal sympathetic discharge and for normal responses of the autonomic centres to external or internal stimuli. In this respect the central sympathetic neurones behave quite differently from the post-synaptic adrenergic neurones, which cease to transmit impulses when their noradrenaline stores have been depleted by reserpine. This difference might be due to the fact that the function of noradrenaline in the brain is not one of transmitter in sympathetic pathways; but it could also be that central adrenergic neurones, if such neurones exist in these pathways, differ so much in their turnover of noradrenaline from the long peripheral nerves that they remain functioning at a low content of transmitter.

In view of the results obtained in this work it seems surprising that, as Bein (1955) observed, a single injection of a small dose of reserpine should arrest all efferent activity in the cardiac nerves. In the cervical sympathetic we saw no disappearance, and only occasionally some temporary diminution, of preganglionic discharge following an injection of reserpine.

In the splanchnic nerves of the cat, Dontas (1957) found no diminution of preganglionic activity unless the dose was above 1 mg/kg. As the technical details of Bein's study have not been reported, it is impossible to decide whether the cardiac nerves respond differently from the cervical sympathetic or splanchnic nerves, or whether the experimental conditions were not comparable.

The fall in blood pressure which occurs soon after a single small intravenous dose of reserpine in animals with a high resting blood pressure has never been satisfactorily explained. It is tempting to suggest that this effect might be caused by the local action of catecholamines released from the region of the carotid sinus and carotid body on receptors in the carotid sinus. Palme (1944) has shown that local application of adrenaline to the tissue around the carotid sinus causes a precipitous fall in blood pressure, which is particularly pronounced in the rabbit but is also seen in the cat. He explains the effect as a stimulation of the carotid nerve by adrenaline, and Landgren, Neil & Zotterman (1952) have indeed demonstrated an increase in the discharge from the carotid nerve when adrenaline or other pressor agents were applied to the exposed carotid sinus of the cat. The effect was quite prolonged, and during that period carotid occlusion produced little or no rise in blood pressure. Impairment of the carotid sinus reflex also occurs after reserpine (Bein, 1953), and it may be that both this phenomenon and the fall in blood pressure have their origin in the action of locally released catecholamines on the carotid sinus.

SUMMARY

1. The action potentials in small strands of the preganglionic cervical sympathetic nerves were recorded in normal and in reserpine-treated cats anaesthetized with chloralose.

2. The activity in the undisturbed normal cat consisted of bursts of impulses, individual fibres contributing one or a few impulses to each burst. The frequency of discharge was often below 1/sec, but occasionally as high as 30/sec.

3. The activity was subject to fluctuations: (1) a rhythm related to the respiratory blood-pressure waves, the discharge being least at each pressure maximum; this activity disappeared when the blood pressure was raised with adrenaline; (2) a rare primary respiratory rhythm, rather insensitive to rise in blood pressure; (3) a cardiac rhythm, in which waxing and waning of activity occurred with each arterial pulse.

4. Asphyxia increased the discharge. Readmission of air into the lungs caused an abrupt standstill of activity, which was resumed as the asphyxial rise in blood pressure subsided.

5. Short acoustic stimuli elicited bursts of impulses with a latency of about 200 msec, the frequency of firing in individual fibres reaching 100/sec.

6. The injection of large doses of morphine was followed by a period of increased efferent activity.

7. After several days of treatment with reserpine (1 mg/kg), the sympathetic activity differed from normal only in the following points: (1) The discharge was more continuous, perhaps more frequent, and lacked a respiratory rhythm as also did the blood pressure. (2) Restoration of the air supply after asphyxia did not arrest the activity; discharge, greatly enhanced over the resting level, continued for as long as the fall in blood pressure which accompanied and followed asphyxia in these animals. (3) Intravenous injections of morphine stimulated the activity less than in the normal cat.

8. Injection of small doses of reserpine (0·1 mg/g) into normal cats in the course of an experiment did not greatly change, and never abolished, sympathetic discharge, irrespective of whether a fall in blood pressure had followed the injection.

9. The conclusion arrived at was that preganglionic sympathetic discharge, both spontaneous and in response to stimuli, was not fundamentally altered by doses of reserpine which abolished all peripheral sympathetic activity and caused severe depletion of the stores of noradrenaline and 5-hydroxytryptamine in the brain.

We are grateful to Ciba Laboratories Ltd., Horsham, for a generous supply of Serpasil.

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THE FINE STRUCTURE OF THE NEUROMUSCULAR JUNCTION OF THE FROG

By R. BIRKS, H. E. HUXLEY and B. KATZ
From the Department of Biophysics, University College London

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In recent years fairly detailed information has been obtained on the physiological events which occur at the vertebrate neuromuscular junction. Experiments on frog and mammalian muscle have shown that the transmitter, acetylcholine, is released from the axon terminals in discrete multi-molecular packets, and attention has been directed to the presence of submicroscopic 'vesicles' inside the nerve endings and their possible significance in the quantal distribution of the transmitter agent (de Robertis & Bennett, 1954, 1955; del Castillo & Katz, 1956; Robertson, 1956*b*; Palay, 1956; de Robertis, 1958; Reger, 1958; Katz, 1958). The purpose of the present work was to examine in more detail the submicroscopic structure of the myoneural junction in the frog from which the greater part of the physiological evidence had been derived. The basic information on the structure of this synapse is already available, as a result of the remarkable microscopic and histochemical studies of Couteaux (1947, 1955, 1958).

METHODS

For most purposes junctional regions of muscle fibres situated at the edge of the frog's sartorius were selected. The muscles were carefully dissected, mounted spirally on Perspex rods and examined in transmitted light with a dissecting microscope, in the way normally used for physiological experimentation (see del Castillo & Katz, 1957*a*). The innervation of the edge fibres of the muscle can be recognized without much difficulty even in the fresh state and shows up more clearly after fixation in osmic acid, enabling one to cut out a small junctional region, about 0·5 mm square, for further examination. The use of fibres on the edge of the muscle has the further advantage of providing suitably thin specimens for fixation and embedding. If potassium permanganate is used as a fixative, previous marking of the required area is necessary, e.g. by attaching a small ligature to the connective tissue at the edge. An alternative method is to dissect a fine bundle of fresh muscle fibres (from the adductor longus) whose innervation zone can be easily recognized. However, selection of junctional portions from the edge of the sartorius was so simple and successful that it was adopted throughout.

The usual procedure was to cool the muscle and then to fix it in ice-cold OsO_4 solution, 1% (w/v), buffered with acetate-veronal (Palade, 1952), for 30 min. After transferring it to 70% ethyl

alcohol, the junctional regions were cut out, dehydrated and stained during the night with 1% phospho-tungstic acid (PTA) in alcohol. Some muscles were fixed in 0·6% buffered $KMnO_4$ solution (Luft, 1956) for 6 hr at 0° C, others in OsO_4 without additional PTA stain. The specimens were embedded, after preliminary soaking for at least 24 hr, in 'Araldite' mixture (Glauert & Glauert, 1958), in gelatine capsules or polythene moulds shaped so that they could be conveniently mounted for longitudinal or transverse sectioning.

The majority of specimens were examined in longitudinal section, which was found to give the more useful information. The disadvantage of this procedure was that, because of the characteristic longitudinal spread of the amphibian nerve endings along the surface of the muscle fibre (Kühne, 1887), more time had to be spent in finding sections which contained them. For final examination in the electron microscope (Siemens, Elmiskop I, used at 60 or 80 kV), the microtome was set to provide sections approximately 400–800 Å thick.

RESULTS

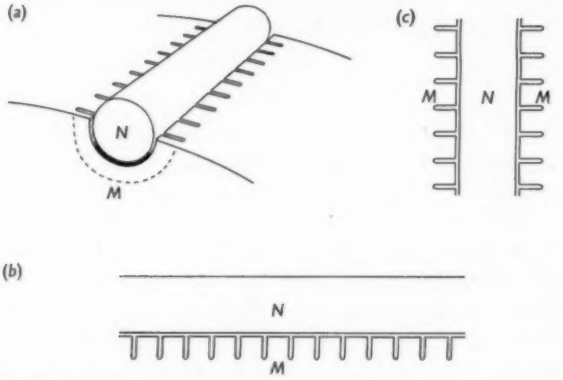
To appreciate the details seen in electron micrographs of the myoneural junction, it is necessary first to give a brief summary of its general lay-out as revealed by optical microscopy. The following description applies to the innervation of 'fast' skeletal muscle fibres in the frog. The myelinated motor axon approaches the muscle fibre and gives off an array of non-myelinated terminal branches which spread along the fibre surface in both directions in the shape of an elongated 'tree' or 'antler' (Kühne, 1887). Its total length may amount to a few hundred micra. The terminal branches run parallel to the axis of the muscle fibre and lie in shallow 'gutters', i.e. depressions formed by the fibre surface. In Text-fig. 1 a diagram of a small part of such a nerve–muscle junction is shown. An additional feature is seen in this diagram, that the superficial gutters of the muscle fibre are depressed at regular intervals into deeper local 'trenches', the so-called junctional folds (Robertson, 1956*b*) which run at a right angle to the fibre axis and communicate with the external space at both sides of the nerve terminal.

It may be recalled that this system of trenches shows up strikingly with specific cholinesterase-staining methods (Couteaux, 1955), and because of its solid appearance was at one time regarded as a palisade-like structure consisting of a series of lamellae, rather than of folds containing, and communicating with, the aqueous extracellular phase. There is, of course, no conflict between this latter view which is based on electron-microscopic examination (Palade, 1954; Robertson, 1956*b*) and the histochemical result, for the structure of the folds cannot be resolved with the light microscope.

In Text-fig. 1 three principal planes of section are illustrated which determine the aspect of the myoneural junction seen in the electron micrograph; a transverse section and two longitudinal sections through the muscle fibre, one of which is called tangential because it grazes the muscle at the level of its junctional folds while the others pass more deeply through the fibre.

Plates 1, 2 and 5 show typical electron micrographs from a myoneural junction of the frog sartorius in longitudinal section through the muscle fibre. If one compares them with the diagram (Text-fig. 1), the most interesting

difference, apart from the wealth of structural detail, is the presence of a third cellular layer which separates the nerve ending from the extraneous connective tissue. This layer is made up of nucleated cells which form the terminal Schwann-cell cover of the axon. Plate 2*A* shows a longitudinal section which passes through the nuclear region of this 'satellite cell'. The triple-complex, Schwann cell, nerve ending, muscle fibre, is seen equally in permanganate-fixed preparations (Pl. 4*A*) and in the transverse sections in Pls. 2*B*, 3 and 4*B*.



Text-fig. 1. Diagram of neuromuscular junction. (*a*) Showing small portion of a terminal axon branch (*N*) lying in a 'gutter' formed by the surface of the muscle fibre (*M*), and the array of semi-circular, post-junctional, folds. The frontal view shows cross-sectional aspect of the junction. The dotted line indicates the recessed border of the muscle fibre in the junctional fold. (*b*) Viewing the junction in longitudinal section; (*c*) in 'tangential' section.

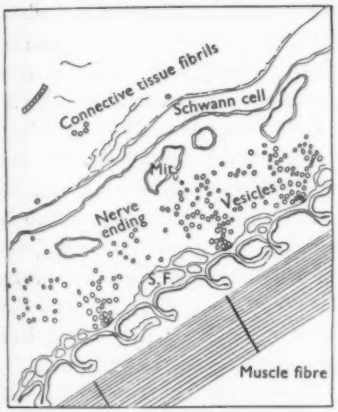
Much of the structural detail shown in the present pictures has been described before (Robertson, 1956*b*; Palay, 1958; Andersson-Cedergren, 1959) in connexion with other neuronal or myoneural junctions, and there is no need here to point out more than a few special features.

Terminal Schwann-cell layer

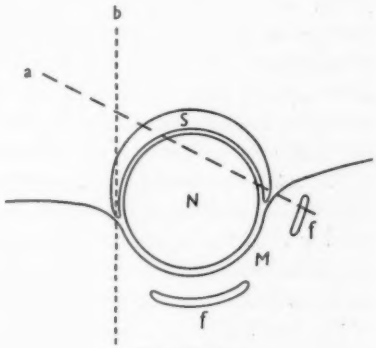
In the majority of the micrographs the Schwann-cell coating, which separates the axon terminal from collagen and finer connective tissue fibrils, was seen quite clearly. In some sections the Schwann cytoplasm formed a very thin layer and its border lines were at times obscured, presumably as a result of oblique sectioning. But in only one out of several hundred micrographs did we find a nerve ending which was 'naked' and clearly lacked a Schwann cover over an appreciable length.

A point of interest is the extent to which the satellite cell intrudes into the synaptic space between nerve and muscle. In cross-section (Pls. 2–4) the

Schwann cell is seen to engulf the nerve ending, but it never surrounds it completely, and its contact with the muscle fibre is restricted to a short stretch at either side of the axon. In longitudinal section (Pls. 1, 2, 5; Text-fig. 2), one often finds cellular segments of various shapes interposed between nerve and muscle. These are probably parts of the same Schwann cytoplasm whose 'fingers' protrude in an irregular way into the synaptic region. It is clear, at any rate, that a longitudinal section through the structures shown transversely in Text-fig. 3 may pass through the Schwann cell twice, and show its edge interposed between nerve and muscle surfaces.



Text-fig. 2



Text-fig. 3

Text-fig. 2. A tracing of some characteristic components of the myoneural junction, from Pl. 1. *Mit.*: mitochondria of axon terminal; *S.F.*: a 'Schwann finger' (cf. Text-fig. 3, line *a*).

Text-fig. 3. Schematic cross-sectional view showing Schwann cell (*S*), nerve ending (*N*), muscle fibre (*M*) with junctional folds (*f*). *a* and *b* indicate planes of longitudinal section in which the Schwann cell may be seen in contact with the muscle fibre (and, in *a*, interposed between nerve and muscle).

It will also be evident that there is a chance of missing the nerve ending and obtaining a longitudinal aspect in which only a layer of Schwann cytoplasm is seen in contact with the synaptic (i.e. folded) region of the muscle membrane. However, such occurrences would be rare, and we have, in fact, never found a section in which the nerve ending did not show up at some place of the junctional region.

In spite of this complication, the use of longitudinal rather than cross-sections has one definite advantage, namely that it enables one to identify the synaptic region in normal as well as denervated muscle by the very regular disposition of the junctional folds which survive nerve degeneration (Birks, Katz & Miledi, 1960). Because of their regular and approximately transverse alignment, junctional folds are much less conspicuous in cross-sections of frog muscle. In this respect the situation in the frog differs from the arrangement in reptiles and mammals, as has been fully explained by Couteaux (1958).

The structural detail of the Schwann cytoplasm varies greatly from one area to the next. In some sections no inclusions can be seen apart from a few fine granules and filaments. In other regions there is an accumulation of 'vesicles' of various sizes and of mitochondria. Occasionally complicated deep foldings of the cell membrane are observed. The nucleus is surrounded by a 'perinuclear bag', a few hundred Ångström units wide, with a thin membrane separating it from the cytoplasm.

Membrane relations

The surface membrane of the Schwann cell shows the characteristics described by Robertson (1959). It is separated from the axon membrane by a space of about 150 Å width (this measurement applies to preparations fixed with OsO_4; in those fixed with $KMnO_4$ (Pl. 4*A*) the space appears to have shrunk considerably). On the other side of the Schwann cell, facing the connective tissue space, there is the familiar external dense layer separated from the Schwann cell membrane by a space *ca.* 200–300 Å across. This layer can be followed around the surface of the Schwann cell up to the place where it contacts the muscle fibre (Pls. 3, 4*B*, 5*B*). At this point, the external layer fuses with a similar dense layer which envelops the muscle membrane. It is noteworthy that this layer can be traced continuously along the whole of the muscle fibre membrane right into the depths of its synaptic folds, while it does *not* penetrate at any point into the spaces between Schwann cell and axon.

The functional significance of this external layer is obscure. It is clearly not an integral part of any individual cell membrane (see also Robertson, 1959), and its intermediate position in the synaptic space between nerve and muscle makes it difficult to suppose that it can act as a diffusion barrier with the properties envisaged for the cell membrane itself. There is some additional evidence which distinguishes this dense layer from the membranes enclosing the individual cells and some of their internal particles. The electron-microscopic density and 'staining properties' of this layer were found, under different treatments, to be closely related to those of the muscle proteins and not those of the cell membrane: for example, in $KMnO_4$-fixed preparations (Pl. 4*A*), neither the myofibrils nor the external layer show up, while the membranes of the three cells, and of the intracellular vesicles, nuclei and mitochondria are observed in good contrast. In other $KMnO_4$-treated specimens the external layer was just visible, though at much less contrast than in OsO_4+PTA preparations; in the same specimens the myofibrils showed up rather more densely than in Pl. 4*A*. Although these observations throw little light on the chemical properties of the external layer, they do at least support the idea that its composition differs from that of the cell membranes, and are not in conflict with what must be postulated on physiological grounds.

Axon terminals

The nerve endings are characterized by well-known cytoplasmic inclusions, notably an accumulation of 'synaptic vesicles' which have been described by many authors (de Robertis & Bennett, 1954, 1955; Palade, 1954; Palay, 1954; Robertson, 1956*b*).

There has been some question (Robertson, 1959) concerning the dimensions of these 'vesicles' and whether they do not really represent a cross-sectional aspect of a system of tubes. Such tubes, however, would have to be highly oriented to give the observed regularity of oval outlines, and their tubular aspect should be revealed by changing the plane of sectioning. In fact, the same population of vesicles is seen independent of the plane of sectioning (e.g. Pl. 2, *A–C*). Furthermore, in thick sections vesicles were seen whose contours overlapped, an observation which cannot be explained on the basis of a tubular structure. Moreover, whenever it was possible to examine serial or near-by sections of one specimen, with a sufficiently sparse vesicular distribution, it was found that the individual vesicles had dropped out in successive slices, while cylindrical structures of similar diameter (e.g. cross-sectioned groups of collagen fibrils) could be traced without difficulty. Nevertheless, on a few occasions numerous short 'tubes' or a meshwork of branching tubes were seen quite apart from mitochondria and vesicles. The diameter of these tubes was somewhat smaller than that of the vesicles (the latter being of the order of 500 Å).

Vesicles were counted in a series of sections from two specimens and their average 'volume density' was estimated on the assumption that each section covered effectively 800 Å in depth. The result was approximately 1000 vesicles/μ^3 of axon terminal. If the whole of one terminal arborization occupies 1000 $\pi\mu^3$ (this being the volume of a cylinder of 1μ radius and 1 mm length), the total number of vesicles present at such a junction would be approximately 3×10^6.

Another typical feature of the axon terminal is its mitochondria. They possess the characteristic twin-membrane of *ca.* 150 Å separation but their internal structure differs in appearance from the muscle mitochondria (Pl. 2*A*) which show a much more regular parallel system of cristae.

Within the terminal axoplasm the different particles are distributed, in general, not at random but in a consistent orderly pattern. Frequently one can divide the nerve terminal longitudinally into two main portions, a 'mitochondrial region' adjacent to the Schwann cover, and a 'vesicular region' near the muscle surface. Even within the latter region one often sees the vesicles concentrated in certain well defined areas (Pls. 1, 2*A* and 5) focused on a dense zone of the axon membrane directly opposite a post-synaptic fold. This interesting feature recurs much too frequently to be dismissed as fortuitous,

and it reminds one of somewhat similar observations on other types of synapses (Palay, 1958; Edwards, Ruska & de Harven, 1958).

The differentiation of the terminal axoplasm is at times made more obvious by the presence of bundles of very fine longitudinal filaments (less than 100 Å thick, with dense spots recurring a few hundred Å apart), which appear to separate the vesicular region from the rest of the axon terminal, for instance in Pl. 2*A*.

Finally some unusual observations should be mentioned which are illustrated in Pl. 6. The nerve terminal shows here an even greater variety of structural detail than is generally found. Some of the 'vesicular islets' (in the upper part of Pl. 6*B*) are probably axon processes which protrude into the Schwann cell. But there are also various concentrations of vesicles within the axon itself, each bounded by a twin membrane; and the smallest of these enclosures are difficult to distinguish from terminal 'mitochondria' whose internal structure has often a tubular or vesicular appearance. Without serial reconstruction it is, of course, not possible to decide whether the various vesicular aggregates are completely enclosed in separate compartments, or formed by complicated invaginations of the axon terminal (whose membrane shows indeed some deep folds, e.g. Pl. 6*A*).

The post-synaptic folds

These have been first described by Palade (1954) and Robertson (1954, 1956*b*), and it is only necessary to point out some peculiarities of the amphibian junction. The longitudinal spacing of the folds is rather regular: approximately 3–4 per sarcomere. The width of the folds varies between about 500 and well over 1000 Å, the central core of their lumen being occupied by the dense external layer discussed on p. 138. The deeper parts of the folds bend and run obliquely with respect to the fibre axis; they often branch and anastomose with adjacent folds.

In cross-section the chances of including a substantial portion of any single fold are not high, and one usually sees only fragments somewhere below the level of the myoneural contact. Occasionally, the section passes through the fold tangentially (e.g. Pl. 4*B*) and then shows a dense post-synaptic patch, and facing it a concentration of vesicles in the nerve terminal lined up against a dense portion of the axon perimeter (cf. p. 139).

The post-synaptic membrane itself is distinguished by an exceptionally high density of its superficial contours. These are always more heavily 'stained' than the ordinary membrane of the rest of the muscle fibre or of the axon or Schwann cell. It is noteworthy that this special contrast shows up very clearly in specimens treated with OsO_4 or with OsO_4 plus PTA, but not in those treated with $KMnO_4$. On the lines of the argument presented on p. 138 this might be taken to indicate the presence of a special protein layer attached to

large parts of the post-synaptic membrane. There is a further interesting differentiation within this area, seen in all OsO_4 + PTA preparations (e.g. Pl. 1), namely that the region of high density does not extend into the deeper parts of the folds; but this could conceivably arise from inadequate penetration of the stain.

DISCUSSION

The fine structure of synapses is of interest in so far as it may be related to physiological events. Unfortunately such relations are difficult to establish experimentally, and their discussion is at present based on little or no direct evidence. Some promising observations have been made by de Robertis (1958), which show that it is possible, at least in certain types of neuronal junctions, to produce significant structural changes by periods of stimulation or imposed inactivity. We have not been successful in obtaining conclusive evidence from muscles which had been subjected to even quite drastic synaptic 'stimulation' (e.g. exposure to high potassium concentrations and hypertonic media).

A reduction in number of the terminal vesicles was seen on some such occasions, but was not convincing because of the wide range observed 'normally'. A more striking change was a swelling and internal break-up of mitochondria in the nerve endings, which, however, could be interpreted as an unspecific sign of cell damage. Much more significant alterations are found during the early stages of neuromuscular degeneration, which will be described in a subsequent paper.

There are certain features of synaptic structure which have figured prominently in recent physiological discussions (see Eccles, 1957; Katz, 1958). Among these are the absence of cytoplasmic continuity, i.e. the completeness of the membrane separation at the synapse; the size of the extracellular space between the contacting cells; and the function of the pre-synaptic vesicles.

These features have been discussed, and plausible roles can be allotted to them, in the light of what is known about chemical transmission at certain synapses. Thus, the presence of separating membranes and an extracellular space between them is more readily compatible with chemical transmission than with direct 'core-conduction' (provided we are right in assuming that the electrical properties of these structures are comparable to those of membranes and interspaces elsewhere). The space of several hundred Å, between nerve and muscle membrane, and between the walls of the junctional folds, communicates with the extracellular fluid (without interposition of a cell membrane). This would constitute an undesirable leakage path for electric transmission from nerve to muscle, while it provides a highly desirable low-resistance path for the spread of depolarization from the post-synaptic focus to the rest of the muscle membrane (see Eccles & Jaeger, 1958; Couteaux & Katz, 1957). The whole of this space, including the dense external layer, was at one time classified as a 'membrane complex' (Robertson, 1956*a*, *b*; modified recently, 1959); but it

should be realized that these are the very spaces to which drugs like ACh and curare must have rapid access when applied from the external medium (del Castillo & Katz, 1957*a*, *b*).

As regards the role of the vesicles, very little can be added to what has been said before. There is good evidence that transmission at the nerve–muscle junction involves the release of ACh from the axon terminal, not as independently diffusing molecules, but in discrete multi-molecular packets (Fatt & Katz, 1952; del Castillo & Katz, 1956; Katz, 1958). The size of these quantal units appears to be quite independent of the state of the axon membrane, and to be determined by some other component of the nerve terminal. It has, therefore, been suggested that ACh may be contained in the vesicles and released in an all-or-none manner when the vesicle collides with certain spots of the terminal membrane. Such an event may involve coalescence of the membranes, of vesicle and axon, and diffusion of the vesicular contents into the synaptic space. It is interesting that the vesicles are frequently concentrated near the region of contact with the muscle fibre, and that they appear to 'converge' on special zones of the axon membrane directly opposite the junctional folds. It would seem an admirable arrangement to have the vesicles queuing for such strategically disposed regions of transmitter release, but it would imply that the intracellular distribution of vesicles is brought about by obscure forces of interaction and not just Brownian movement.

If we were not prejudiced by any physiological evidence, we might be tempted to interpret the structural picture in a very different way; for a simpler assumption would be that the vesicles distribute themselves by random motion from their sites of origin. On purely morphological grounds it might therefore be more natural to suppose that the particles are formed by vesiculation at the dense zone of the axon surface, and then spread within the axoplasm by random motion, until they collide, and possibly fuse, with obstacles, e.g. mitochondria, the axon boundary, or a meshwork of filaments. The purpose of this alternative proposition is merely to emphasize that the morphological picture in itself provides no evidence in support of our ideas, though it may quite easily be fitted to them.

Finally, the question may be raised of what the chances are of observing vesicles in the process of 'discharge'. The answer is not at all encouraging: on arrival of an impulse, i.e. at the highest peak of activity, a few hundred units of transmitter are released at one cell junction. The total length of the arborization within one junction is of the order of one mm, so that a single unit-release would occur, on the average, at points 3μ apart. Thus, even if it were possible to fix the specimen at the peak of synaptic activity, the chances of finding a 'discharging vesicle' are very small, and it would be a hopeless task to identify it. On rare occasions one does observe Ω-shaped structures ('opened flasks') on the synaptic surface of the axon; they might represent broken vesicles but could equally well be connected with the formation of new vesicles, or vacuoles at the fibre surface. Phenomena of this kind are seen much more

frequently at the surface of Schwann and endothelial cells. In effect, the statistical properties of the process of transmitter release are such as to make the chances of its detection in the electron-microscope extremely low.

SUMMARY

1. The fine structure of the myoneural junction in fast skeletal muscles of the frog has been examined in the electron microscope.

2. Three cell layers are found at the junction: (i) a nucleated Schwann cell which covers the 'non-synaptic' surface of the nerve terminal and separates it from extraneous connective tissue; (ii) the axon terminal with its accumulation of 'mitochondria' and '500 Å vesicles'; (iii) the muscle fibre with its regular transverse array of synaptic folds.

3. The 'synaptic space' between nerve and muscle membranes contains a dense intermediate layer which follows closely the contours of the muscle surface. Using different methods of fixation and staining (OsO_4, $KMnO_4$), the relative density of this layer is found to vary in a manner quite unlike that of cellular or intracellular membranes.

4. Within the nerve terminal, mitochondria and vesicles are often concentrated in separate regions. Vesicles tend to accumulate in the neighbourhood of discrete zones of the axon surface directly adjacent to post-synaptic folds.

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EXPLANATION OF PLATES

With the exception of Pl. 4*A*, all micrographs were obtained from specimens fixed in OsO_4 and additionally stained with PTA in alcohol. All scale marks represent 1μ. Plates 1–5 show junctions from frog's sartorius, Pl. 6 from m. adductor longus.

Pl. 1. Longitudinal section through neuromuscular junction. Some details have been traced in Text-fig. 2.

Pl. 2*A*. Longitudinal section passing through nuclear region of Schwann cell (Schw.: Schwann cytoplasm; Schw.n.: Schwann nucleus). *B*. Transverse section. *C*. 'Tangential' section (cf. Text-fig. 1*c*): starting at the top the plane of sectioning passed progressively more deeply into the muscle fibre, so that the axon terminal is seen only in the upper part of the figure, while in the lower part the deeper regions of the junctional folds are still visible.

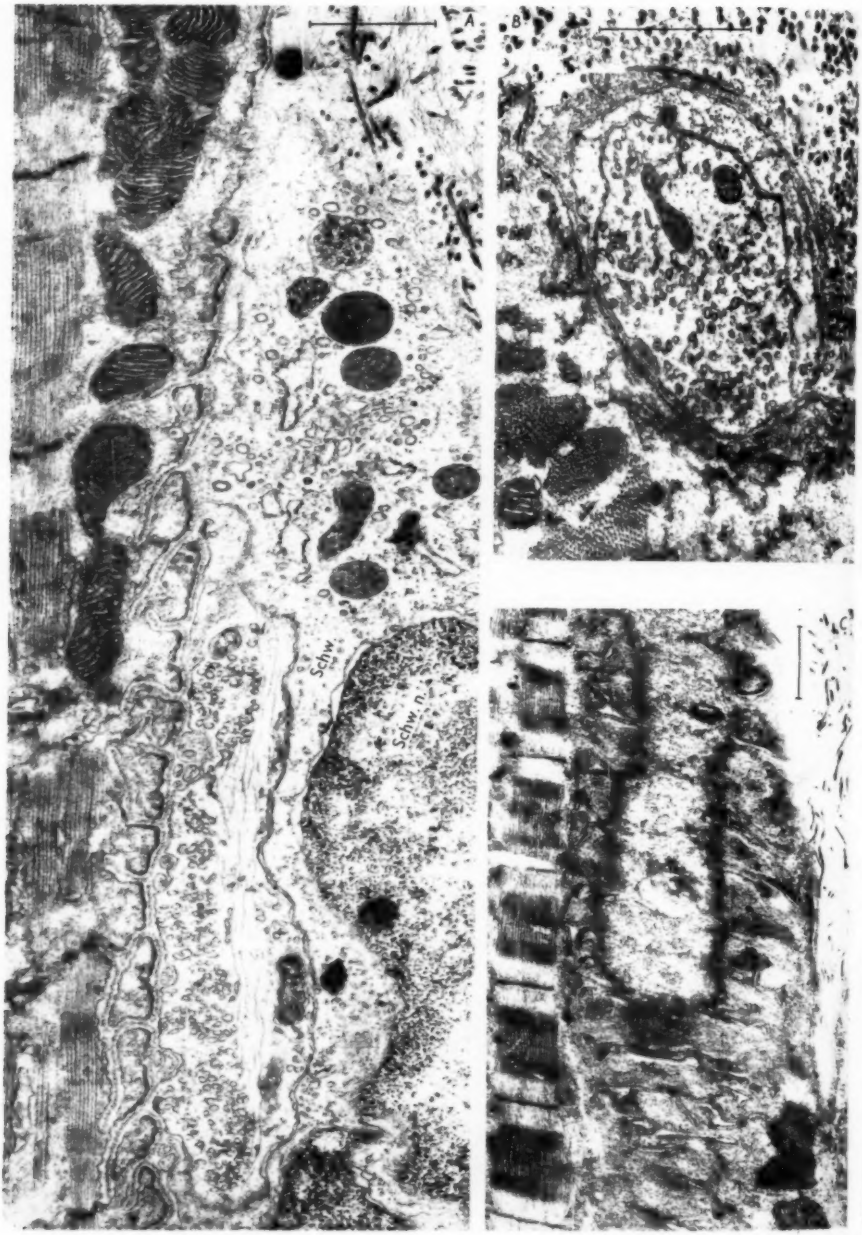
Pl. 3. Transverse sections. In *A*, the Schwann nucleus is seen in the top right part; a 'finger' of Schwann cytoplasm protrudes, from the right, into the synaptic region. Magnifications of *A* and *B* are identical.

Pl. 4*A*. $KMnO_4$-treated muscle. Longitudinal section, showing Schwann layer, vesiculated nerve ending and post-junctional folds. *B*. OsO_4 + PTA. Transverse section passing 'tangentially' through a junctional fold (dark area under the nerve ending).

Pl. 5. Longitudinal sections. Special points: accumulation of presynaptic vesicles opposite to junctional folds in *A* and *B*; in *B*, Schwann cell with nucleus (S.n.) and a 'finger' (s.f.) protruding from the right; c.t. connective tissue space.

Pl. 6. Longitudinal sections. M. add. longus. Note: variety of membrane-bound compartments and vesicular aggregates in the axon terminal.

(*Facing p.* 144)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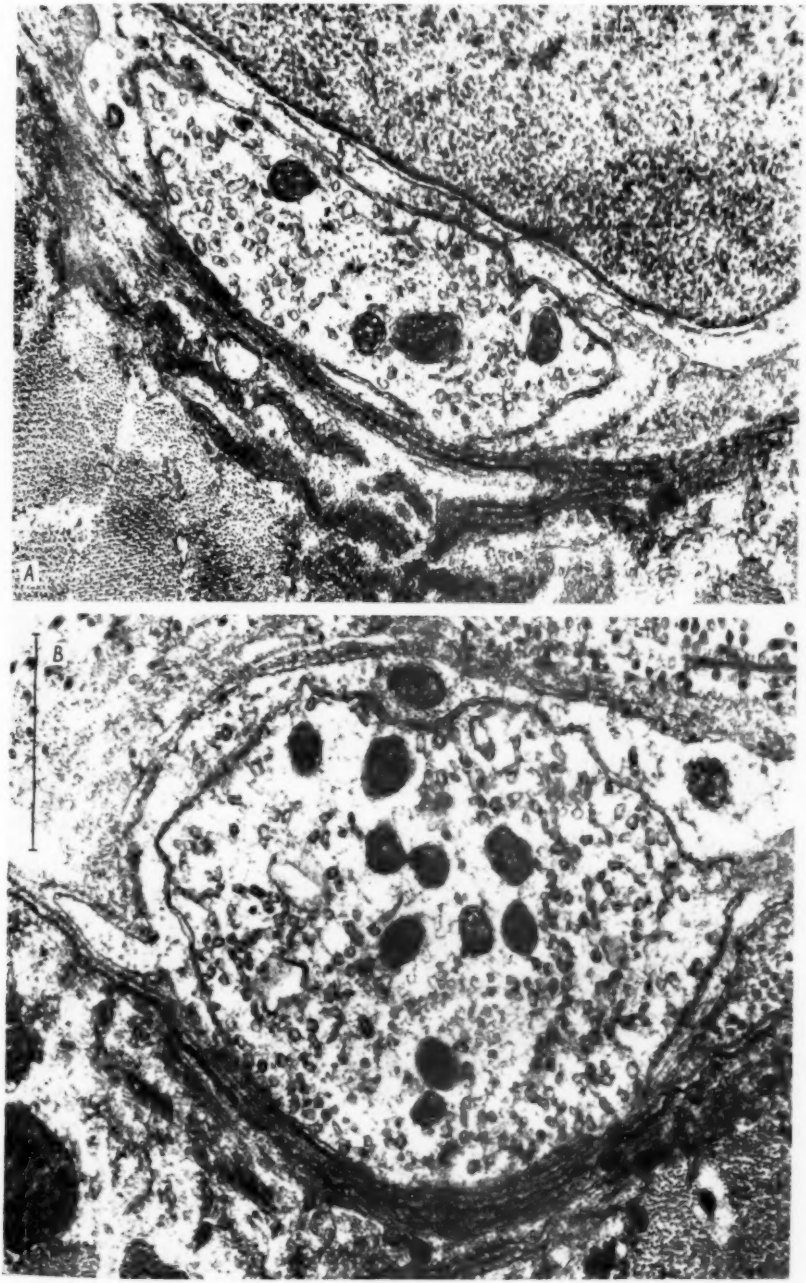
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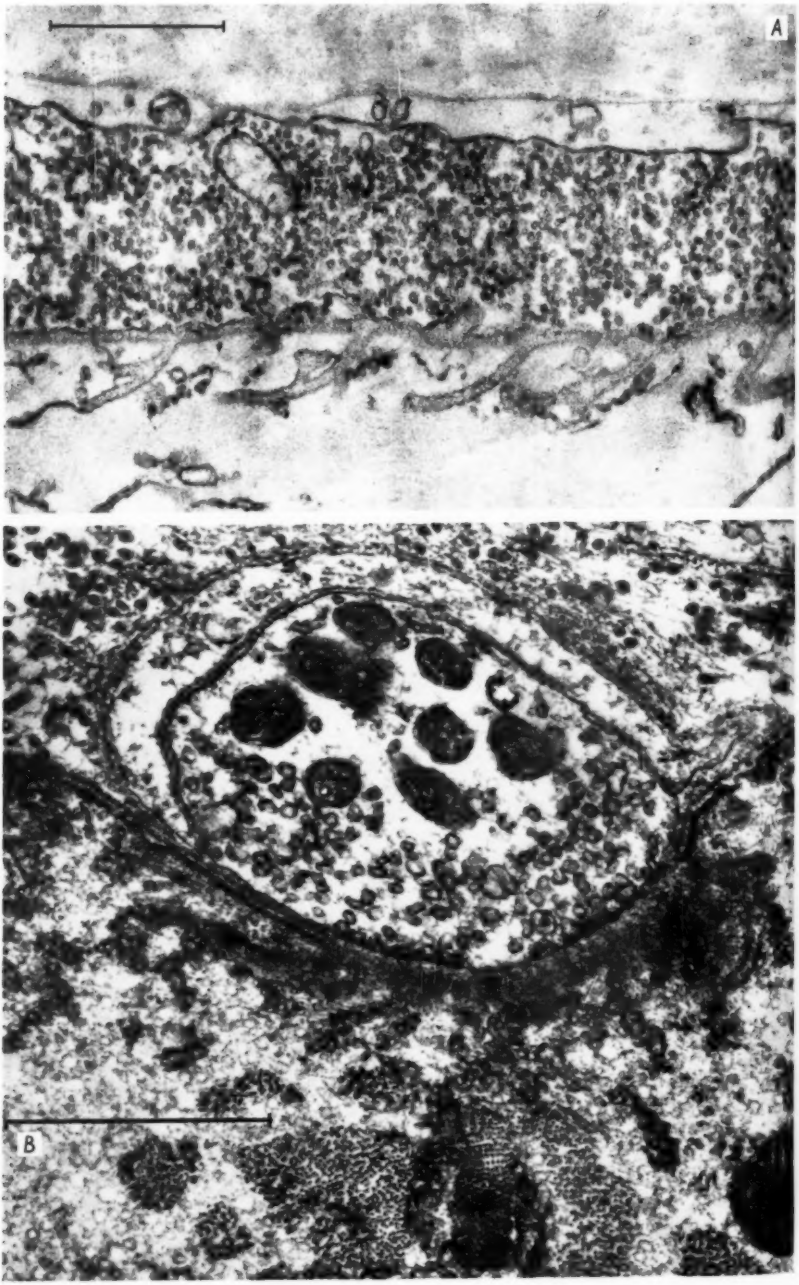
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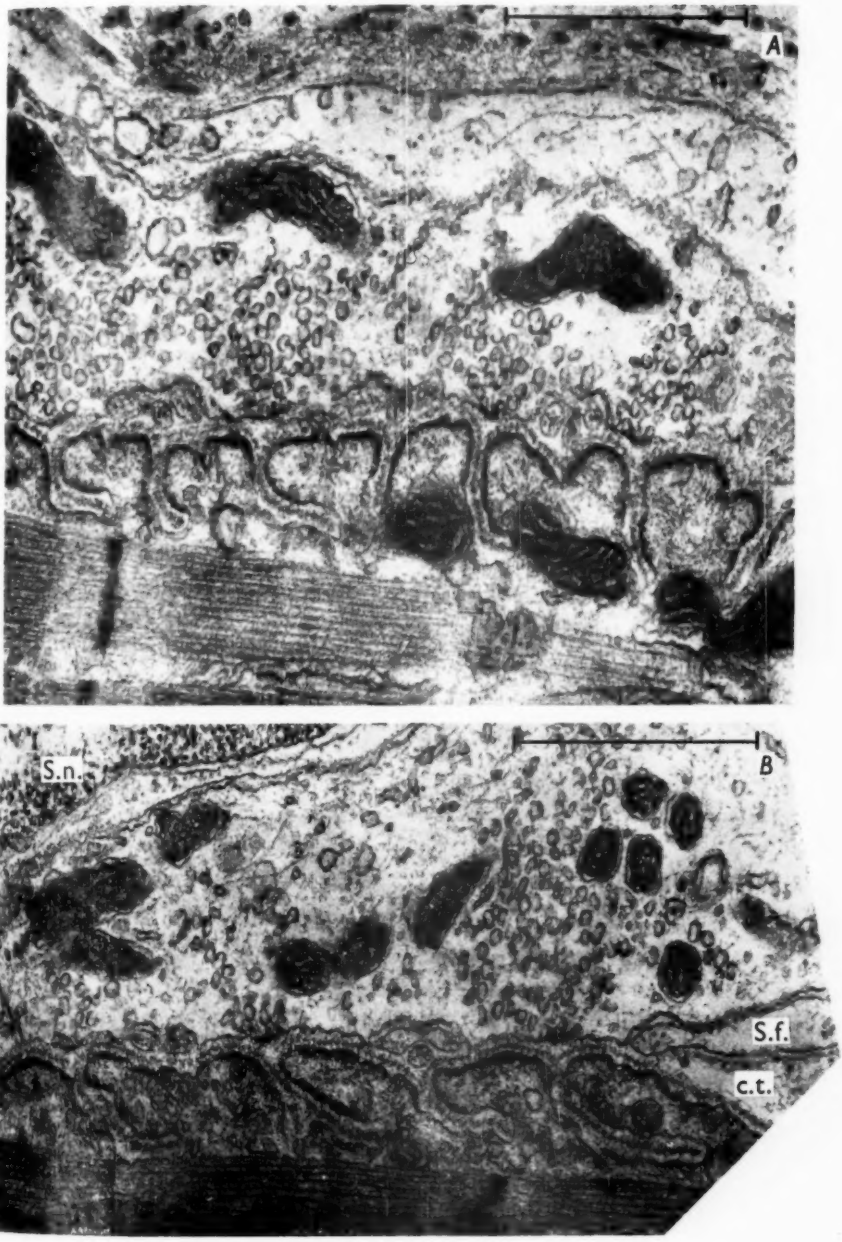
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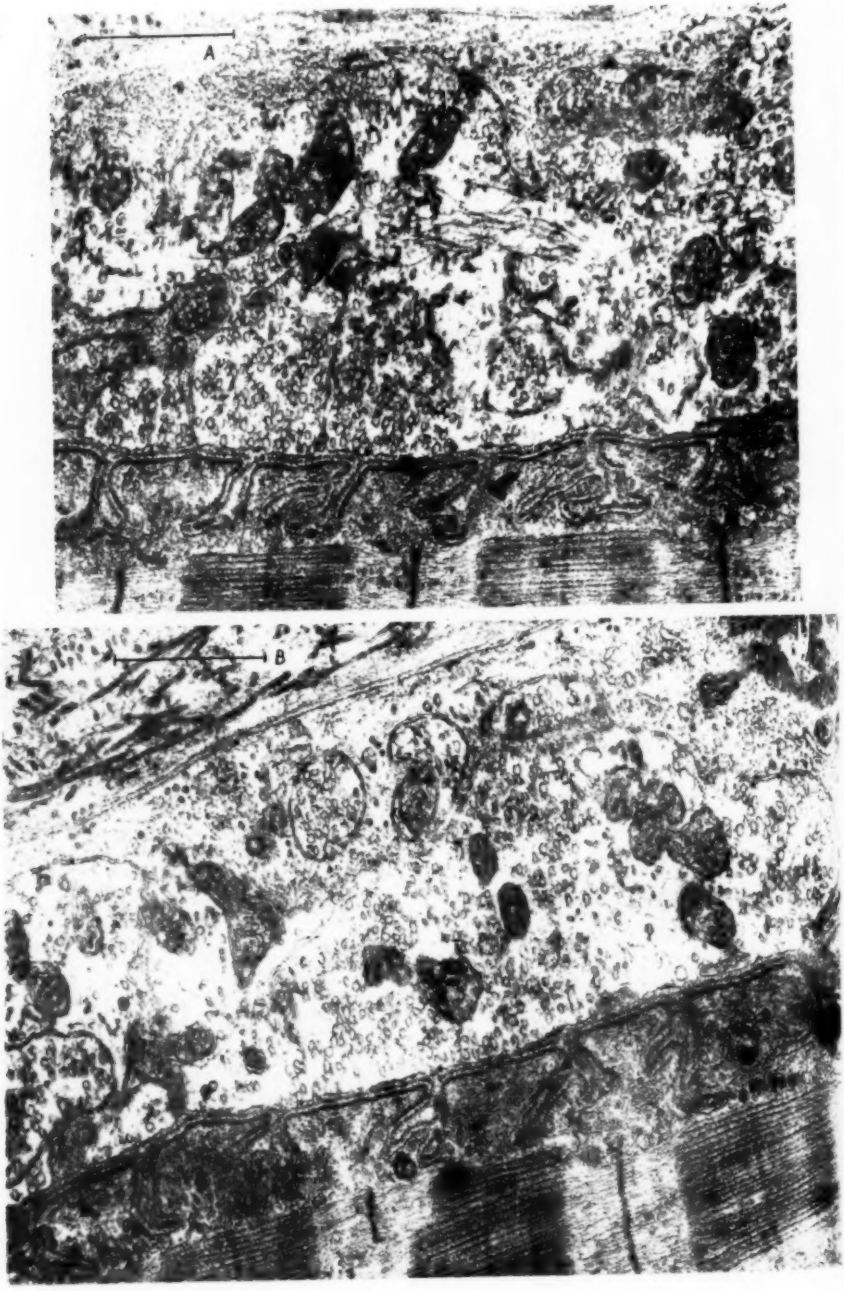
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PHYSIOLOGICAL AND STRUCTURAL CHANGES AT THE AMPHIBIAN MYONEURAL JUNCTION, IN THE COURSE OF NERVE DEGENERATION

By R. BIRKS, B. KATZ and R. MILEDI
From the Department of Biophysics, University College London

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The present work was started in the hope of obtaining further clues as to the function of the 'vesicles' accumulated inside motor nerve endings (de Robertis & Bennett, 1954; Robertson, 1956; Birks, Huxley & Katz, 1960). It was thought that a study of the progressive alterations in function and fine structure, which occur after motor nerve section, might throw some light on the parts played by the different cellular components. Although this initial purpose was not fulfilled, an interesting relation was found between changes in the spontaneous electric activity of the end-plate and certain rearrangements of the presynaptic structures.

It has been known for several years that two kinds of synaptic activity can be recorded from the junctional region of the muscle fibre: (i) the local depolarization produced by a nerve impulse (the end-plate potential, e.p.p.), which normally leads to the propagation of a muscle spike, and (ii) a spontaneous form of activity, consisting of subthreshold 'miniature e.p.p.'s' which occur at random intervals, in the absence of any stimulation (Fatt & Katz, 1952). There is good evidence, reviewed by del Castillo & Katz (1956) and Katz (1958) that the spontaneous discharge results from an intermittent release of multimolecular packets of acetylcholine (ACh) from the nerve terminals. The two types of activity are closely related, for the frequency of the spontaneous discharge is controlled by the membrane potential of the axon terminal (del Castillo & Katz, 1954; Liley, 1956*b*). The frequency increases with progressive depolarization of the nerve endings, and the intense brief depolarization which accompanies the arrival of the nerve impulse causes the discharge of a few hundred units to be synchronized within a fraction of a millisecond, thus producing the normal end-plate potential.

One of the reasons for placing the origin of the spontaneous activity within the nerve endings was the disappearance of the miniature potentials after

nerve section. We have confirmed this observation, but on further study we found that the state of electric silence is only temporary and after the lapse of a somewhat longer period, low-rate spontaneous activity is resumed at the majority of the 'denervated end-plates'. This unexpected finding was pursued in detail with a combination of physiological and electron-microscopic methods. The results will be described in two separate parts, preliminary accounts of which have already been given (Katz & Miledi, 1959*a*; Birks, Katz & Miledi, 1959).

PART I. Electrophysiological study

METHODS

Most experiments were made on paired sartorius muscles of frogs (*R. temporaria*) one of whose sciatic nerves had been severed previously. For some long-term denervation experiments the operation was repeated after about 1 month, when the spinal nerves also were resected to prevent regeneration. Frogs were operated on in batches and kept either at room temperature, or in a cold store at 4–6° C.

In the isolated muscles residual responses to nerve stimulation were studied in the early stages, but our main attention was focused on the recording of spontaneous subthreshold potential changes in the muscle fibres. In several experiments the sensitivity of the muscle to ACh was also tested. The usual procedure was to mount denervated and control muscles side-by-side in the same bath of Ringer's solution, and to record with intracellular electrodes from the fibres. In some cases, especially when the effects of different solutions were studied, samples of spontaneous activity of both muscles were recorded simultaneously.

It was essential to be able to locate junctional regions on many muscle fibres without having the physiological response available as a guide. For some weeks after nerve section this presents no great difficulty, for with a suitable dissecting microscope the innervation of the fibres can still be seen. The success of this visual method of location can be assessed from the fact that, in the control muscles, 90% of the regions selected in this way for the insertion of the micro-electrode showed miniature potentials. These were not all 'focal'; that is to say, one could sometimes obtain a larger amplitude by small subsequent displacements of the electrode, but this was not of great importance for most of the experiments. To aid in the detection of miniature potentials, neostigmine was usually added (approximately 10^{-6} (w/v)) which increases the size and duration of the potentials. There were additional methods of confirming the identification of the junctional regions: tests for local sensitivity to ACh and, after the experiment, fixation and staining by osmic acid and finally examination with the electron microscope.

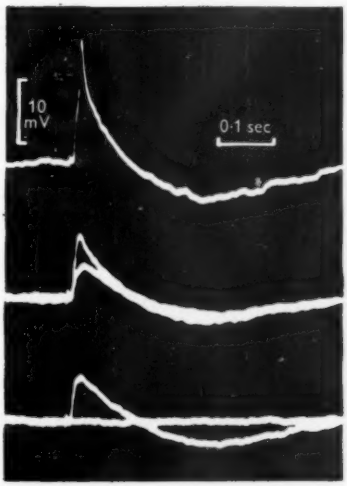
After more than 2 months, however, the intramuscular nerve twigs were no longer visible, and the selection of denervated end-plates became much more difficult and uncertain. The results of long-term experiments were, therefore, less numerous and decisive than those obtained during earlier stages of degeneration. The details of the procedure, involving the use of micro-pipettes for recording, and occasionally for iontophoretic application of ACh, have been fully described before (e.g. Fatt & Katz, 1951; del Castillo & Katz, 1955, 1957).

RESULTS

Failure of transmission and spontaneous activity

Neuromuscular transmission, as is well known, fails at a time when the majority of the severed axons are still capable of conducting impulses. Junctional failure has been reported to occur at very different intervals after nerve section (Sokolow, 1874; Cipollone, 1897; Langley, 1909). There are

several factors responsible for this variation, the most important being the temperature at which the frogs are kept. During a warm spell, with room temperatures around 25° C, the sartorius lost its indirect excitability 3–4 days after nerve section, but in a batch of frogs kept at 5° C it took about ten times longer. There are also characteristic differences between different limb muscles: the indirect twitch disappears in the thigh some days earlier than in the lower parts of the hind leg; in a given batch of animals, a residual twitch could be seen in the gastrocnemius or tibialis anterior for a week after the response had completely ceased in the sartorius.



Text-fig. 1. Intracellular recording from a slow muscle fibre of iliofibularis, 9 days after nerve section. Transmission to the 'twitch' fibres of this muscle had failed, and no e.p.p.'s could be recorded from them. Electric responses to single nerve shocks of different intensities (increasing from below). The responses show the characteristics of 'slow-junction potentials' (Kuffler & Vaughan Williams, 1953; diphasic shape, additive contributions by several nerve fibres).

A rather interesting difference concerns the 'twitch' and 'slow' systems within any one muscle (iliofibularis or ext. l. dig. IV, for instance; see Kuffler & Vaughan Williams, 1953). The indirect response of the 'slow' muscle fibres, evoked by stimulation of small motor axons, invariably survived for a few days after the large axon had failed to excite the twitch fibres (see Katz & Miledi, 1959*b*). Text-figure 1 shows intracellular records from a slow muscle fibre during this period: by grading the intensity of the stimulus, two or three steps of slow-junction potentials could be elicited. The maximum amplitude of the local electric response was about 18 mV, which is comparable to that obtained in normally innervated fibres (Kuffler & Vaughan Williams, 1953).

All this indicates that the transmission at the small-fibre junctions was still intact, although all the large-axon endings had ceased to function and did not even produce a subliminal end-plate potential.

This sharp differentiation, in one muscle, between functionally normal and completely disintegrated junctions was of interest. It suggested that the synaptic failure at individual junctions is not a gradual process in which the safety margin slowly declines, but a sudden break-down after a long latency. This was amply confirmed when we tried to follow the development of the failure among the twitch fibres of the sartorius themselves.

We expected to find a transitional stage in which the response at a large number of end-plates was reduced to the level of a subthreshold e.p.p., but not completely abolished. We have looked for such a phenomenon in several batches of frogs, but without success. What we found, instead, was that a few junctions were still functioning apparently normally, with large e.p.p.'s and spikes and the usual background of spontaneous activity, while at many other end-plates of the same muscle there was no trace of even a residual e.p.p., nor of any spontaneous miniature potentials.

This statement must be qualified in one respect: the residual transmitting junctions tended to deteriorate in the course of the experiment and to fail as a result of repeated stimulation much more readily than normal junctions. During this acute failure subthreshold e.p.p.'s were sometimes observed, and the discharge of spontaneous miniature e.p.p.'s survived the stoppage of the impulse.

In two cases the denervated muscle happened to be removed from the frog at a rather critical time, when its indirect response appeared still quite normal immediately after dissection, but a few hours later had either been reduced to a just noticeable twitch or had completely vanished. At the beginning of such an experiment most or all the end-plates showed spontaneous miniature potentials at the usual range of sizes and frequencies; at the end, few or none could be detected.

An attempt was made to follow the progress of the acute change in detail by intracellular recording of the spontaneous activity. For this purpose a batch of denervated frogs was kept at about 5° C for approximately 3 weeks, so as to slow down and spread out the preliminary stage of degeneration. Acute observations were then made on the isolated sartorius at 20–25° C. Failure of transmission occurred at many end-plates during the experiments. The changes in the spontaneous activity were complicated, but the most striking feature was an ultimate decline in rate, down to a level of a few discharges per minute or less. This was preceded at some junctions by a period of high-frequency discharge, such as might result from a rapid depolarization of the axon terminal. The average amplitude of the potentials also diminished during the experiment, to about one half of the initial size, though occasionally a few large spontaneous potentials of the order of several millivolts stood out among the rest. In spite of their complexities the observations confirmed that at the time of failure of transmission a rapid functional break-down occurs in the nerve endings, associated with a decline in frequency and ultimate disappearance of the spontaneous potentials.

Thus, after a preliminary period of several days during which no gross abnormalities can be observed, neuromuscular transmission ceases probably within hours, and the spontaneous activity also stops. There is little doubt that the changes are entirely due to failure of transmitter release from the presynaptic terminals, for the sensitivity to applied acetylcholine (observing twitch responses as well as local depolarization) was found at this stage to be approximately the same in denervated and control muscles, and at no phase of degeneration have we encountered a reduced sensitivity on the denervated side. We cannot say in what order the terminal apparatus disintegrates: it might start with a depolarization of the terminal axon membrane and a consequent blockage of propagation into the endings. It was possible occasionally, to pick up axon spikes from intramuscular nerve branches quite close to a completely 'silent' end-plate, but this observation is still compatible with a blockage more distally, in the non-myelinated arborization of the nerve. At any rate, whether the transmission failure arises from a local all-or-none blockage of this type or not, the drastic decline of the spontaneous discharge indicates that, within a short period, the whole of the terminal apparatus must be involved.

Resumption of spontaneous subthreshold activity at denervated end-plates

The evidence so far presented is in line with the accepted view (MacIntosh, 1938; Lissák, Dempsey & Rosenblueth, 1939; Feldberg, 1943; Banister & Scrase, 1950), namely that failure of transmission at a degenerating synapse is associated with a stoppage of transmitter production and therefore also with disappearance of the spontaneous miniature potentials (Fatt & Katz, 1952; Liley, 1956*a*). On further exploration, however, we found that spontaneous subthreshold activity was resumed at the denervated end-plates during the course of the second week.

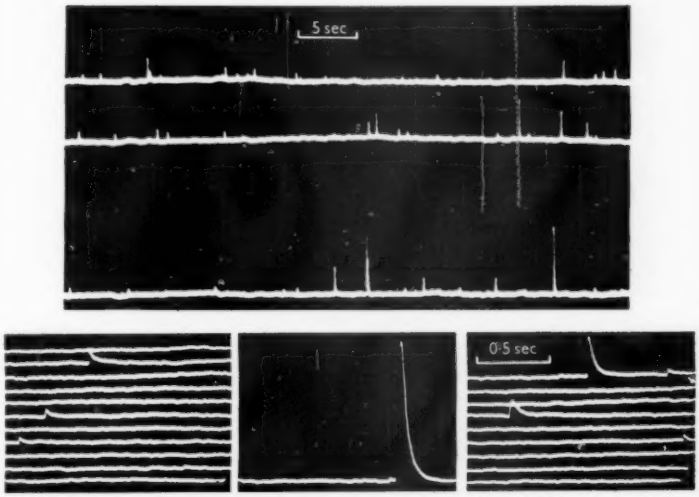
The frequency of this renewed discharge is usually very low, much less than at innervated end-plates, and this made it necessary to alter our procedure. Normally one has to watch the oscilloscope for only 10–15 sec to ascertain whether spontaneous potentials occur at any given locus; in the denervated muscle fibres we adopted the rule of waiting for at least 2 min before withdrawing the micro-electrode. Once the low-rate activity had been detected, care was taken to check the genuineness of the 'silent period' during the earlier phase of degeneration. In some of the batches, observation periods of up to 10 min per fibre were used, and attempts were made by local 'prodding' with a micropipette and by changing the tonicity of the medium (see p. 153) to stimulate any latent activity which might be there, but the results were unchanged: nerve section was succeeded first by disappearance and later by resumption of spontaneous end-plate activity.

In several respects the spontaneous potentials resemble those recorded at normal end-plates. Their location is confined to the junctional region of the fibres, i.e. the places where remnants of the myelinated axons can be seen to terminate and where local application of ACh is most effective (cf. Text-fig. 5). They were never observed at the nerve-free pelvic end. The pharmacological reactions of the local potentials are similar to those of ordinary miniature e.p.p.'s: they are reduced in amplitude or abolished by the usual blocking dose of curarine and increased in size by neostigmine. The 'potentiating' effect of neostigmine was apparently less marked than on the normal muscle, but it was quite definite when tested during the first few weeks of degeneration. In some of the long-term experiments neostigmine appeared to be without effect, but this may well be related to the gradual reduction of cholinesterase activity which has been reported (Couteaux, 1955).

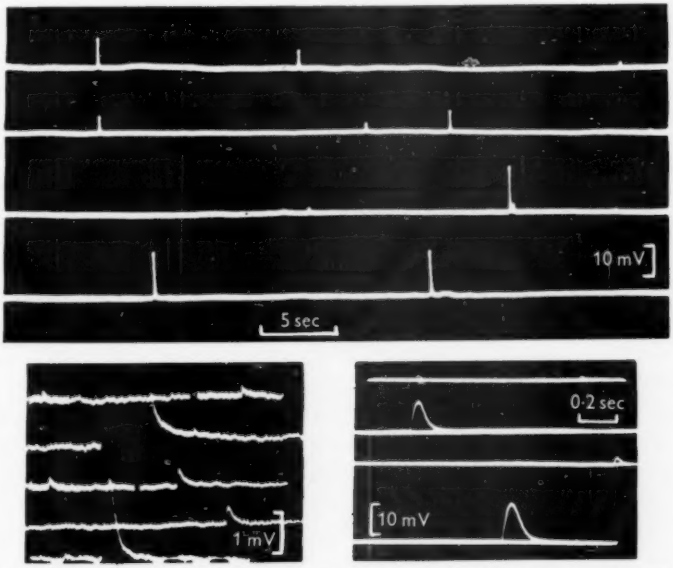
There are, on the other hand, certain consistent differences between the spontaneous activity at normal and denervated end-plates. The low frequency has already been mentioned. The frequency ranges are very wide (see Fatt & Katz, 1952), in denervated as well as control muscles, and overlap to some extent. Normal rates are usually grouped around one or a few per second, though variations between 1 per 10 seconds and over 50 per second have been encountered (the upper values probably resulting from local axon damage). In denervated muscle the rates are some 10 to 100 times lower: roughly the same numbers of discharges can be seen if one waits for minutes rather than seconds. With a random sequence of such long intervals a systematic study becomes very tedious, and most of the quantitative observations were therefore made on the few fibres in which the average frequency exceeded 10 per minute.

Size and time course. In the majority of the experiments neostigmine (10^{-6} (w/v)) was used; this serves to increase amplitude and duration of the normal miniature potentials (Fatt & Katz, 1952). With the exception of a few experiments, no attempt was made to achieve an optimum location of the recording electrode other than using the course of the intramuscular axon branches as a guide.

The *average* amplitude of the potentials was usually about $\frac{1}{4}$ to $\frac{1}{2}$ mV, which is within the range obtained normally (Katz & Thesleff, 1957). However, the amplitude distribution differed quite consistently (Text-figs. 2–4). Instead of the 'Gaussian', bell-shaped distribution found at normal end-plates, we obtain a highly asymmetrical histogram whose maximum is at or near the origin (it usually suffers an underestimate because in the lowest-amplitude class some potentials are bound to be overlooked). The dispersion of sizes is much wider than in normal muscle. There are a few exceptionally large potentials, of about 10 mV amplitude, but this by itself is not a special feature of the denervated muscle: it also occurs at normal junctions, though only infrequently (cf. Liley, 1957).



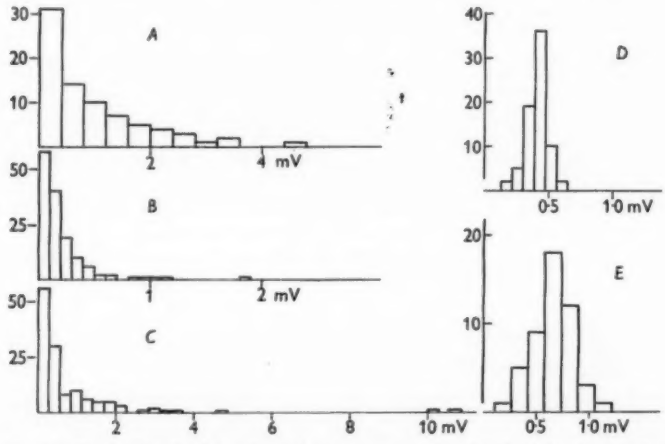
Text-fig. 2. Spontaneous activity at a denervated end-plate. M.ext.l.dig.IV, 9 days after nerve section. No response to nerve stimulation. Upper part, slow continuous recording; lower part, swept base lines, separated by 1 mV deflexions; note the wide dispersion of amplitudes.



Text-fig. 3. Spontaneous activity from a sartorius end-plate. 5 days denervation; no response to nerve stimulation at this end-plate. Upper part, continuous slow recording (note the almost identical size of the three largest potentials, which were also the largest recorded in any experiment); lower part, time base sweeps, two different amplifications; note concurrent variations in amplitude and rise time of individual potentials.

Among the largest potentials there is sometimes a peculiar recurrence of 'preferred amplitudes', as is illustrated in Text-fig. 3. A possible explanation of this phenomenon is that the discharge of large packets of ACh may lead to a saturation of receptor areas, e.g. of the junctional folds nearest to the point of release.

The miniature potentials in denervated muscle were, on the whole, slower and of more variable time course than focally recorded potentials at normal end-plates. Unfortunately no accurate comparison was possible because the very low frequency of the discharge in most cases prevented a definite focal

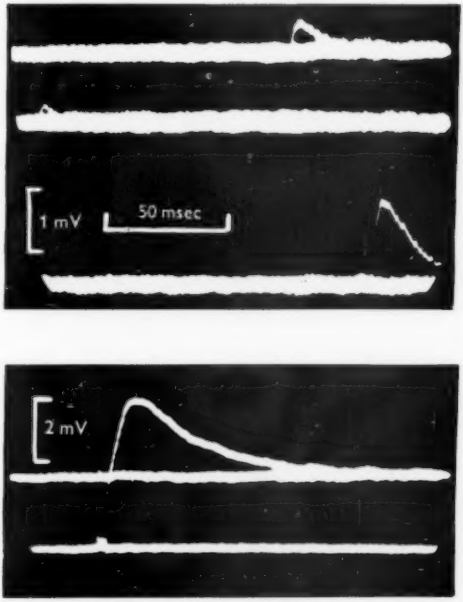


Text-fig. 4. Amplitude distribution of spontaneous potentials. On the left, three end-plates of denervated muscles, *A* and *B* 15 days, *C* 9 days after nerve section; there was no response to nerve stimulation in these muscles. On the right, *D*, an innervated control muscle; *E*, from the only junction still transmitting which was found in a 7-day denervated muscle.

location of the recording electrode. The shortest rise-times were 2·5–5 msec (with prostigmine present, e.g. Text-fig. 5), the longest—observed during exceptionally *large* potentials—were of the order of 50 msec (e.g. Text-fig. 3). To give an approximate estimate, the time course appeared to be some 2–3 times slower in denervated than in normal muscle. Nevertheless many of the rise-times were fast enough to rule out any mechanism which would involve long-range diffusion of a depolarizing agent, from a source more than some micra away.

Agents affecting the discharge rate. One of the most significant features of the normal miniature e.p.p.'s is the sensitivity of their discharge rate to experimental alterations. Any form of stimulus, electrical, chemical or mechanical, which acts on the nerve endings, is apt to produce an outburst of discharges, and it is clear that there is an enormous margin, of the order of a million times, between the 'resting' and the maximum firing rate of the miniature potentials.

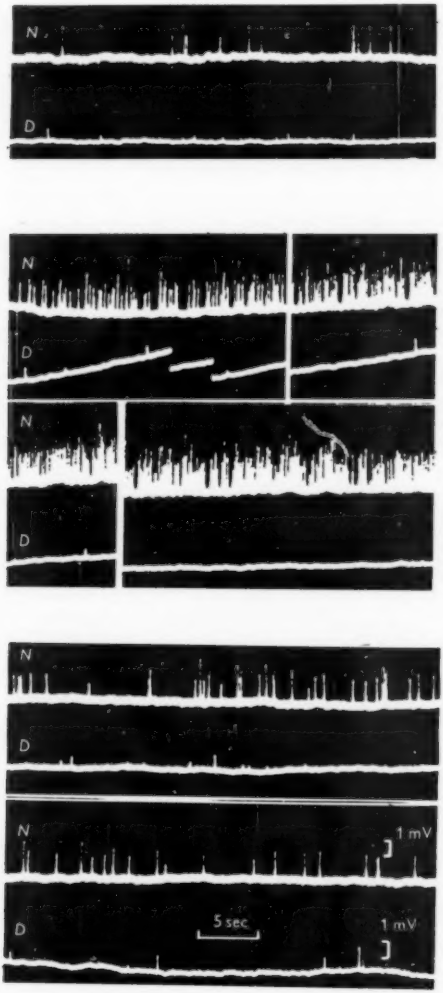
We tried to stimulate the activity at the denervated end-plates by a number of methods which are effective normally, mechanical prodding of the active region with the micro-electrode, application of excess potassium, and of hypertonic solutions (Fatt & Katz, 1952; Liley, 1956*a*; Furshpan, 1956). None of these procedures worked (e.g. Text-figs. 6–7). When a K-rich solution was run into the chamber, the muscle fibres became partially depolarized and the amplitudes of the miniature potentials diminished. While the frequency of the discharge in the normal control muscle increased markedly, there was no apparent change of frequency at the denervated end-plates (Text-fig. 7).



Text-fig. 5. Upper part, time course of spontaneous potentials from a 20-day denervated end-plate; neostigmine 10^{-6} (w/v). Lower part shows an ACh potential produced by a brief iontophoretic pulse (lower trace) from a nearby micropipette.

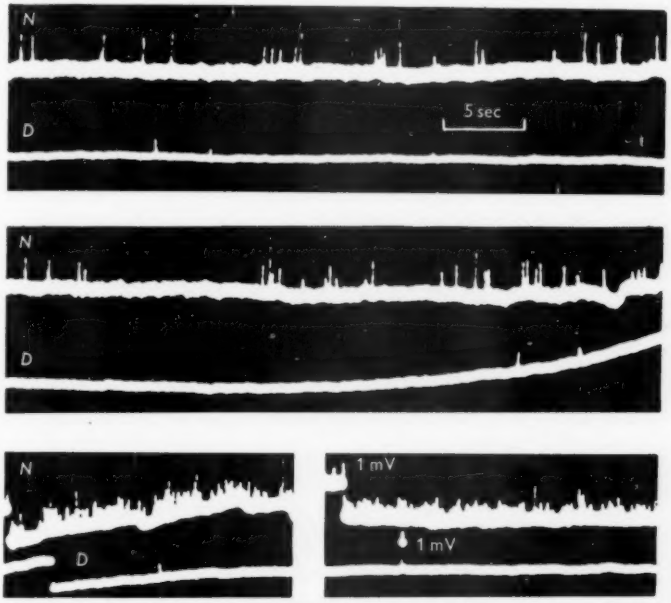
The effect of hypertonic solutions, which is particularly striking at normal end-plates, was studied fairly extensively. Usually the tonicity of the bath was doubled by adding sucrose; in some experiments inulin was used to increase the osmolarity by about 50%. The discharge rate at the normal end-plates went up to well over 100/sec, while we observed no definite increase at any of the denervated end-plates, and at some the frequency was even reduced (e.g. Text-fig. 6). The only exceptions were seen in muscles in which partial regeneration had occurred. In these preparations some end-plates were found

whose miniature potentials resembled the normal, in rate, amplitude distribution and response to hypertonicity, even though nerve–muscle transmission had not yet been re-established (electron-microscopic examination showed that these muscles contained regenerated nerves and endings of normal appearance).



Text-fig. 6. Effect of hypertonic solution on normal (*N*) and 17-day denervated (*D*) end-plate. Top part normal Ringer's solution; middle, solution made hypertonic by adding approx. 7% sucrose; bottom, returned to normal Ringer's solution.

The only agent which was found to produce a significant increase in the frequency of discharge at denervated end-plates was a *reduction* of tonicity. With a Ringer's solution diluted to half strength the rate of firing was raised about tenfold, and at some end-plates reached values around 1/sec. It had previously been observed (Fatt & Katz, 1952; Boyd & Martin, 1956) that, in *normal* muscle the frequency of the spontaneous discharge is decreased by hypotonic solutions; but we found that the effect is more complicated, and

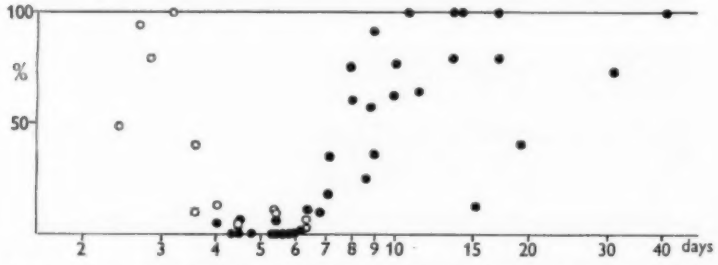


Text-fig. 7. Effect of excess K on normal (*N*) and denervated (*D*) end-plate; cold-stored frog, 104 days denervation. K-rich solution was applied during the middle row, and its effect is seen by slow depolarization and increased frequency of discharge at normal end-plate.

that an immediate fall in frequency (which is striking only if the initial rate was high) is followed after 1–2 min by irregular bursts of high-rate discharges. It appeared, from a comparison of simultaneous recordings, that the delayed action of the tonicity change still occurs at denervated end-plates, while the initial effect (which is dominant in normal muscle) has disappeared.

Having described several distinguishing properties, it is worth while to repeat that in other and, we think, more basic aspects (microscopic location, sensitivity to specific drugs), the spontaneous potentials of normal and denervated muscle represent a very similar form of activity. One of the most puzzling features remains the temporary silent period, which suggests a renewal, rather than simply a residue, of a normal form of activity.

This gap is illustrated graphically in Text-fig. 8, in which results from six batches of frogs (46 muscles) have been pooled. As there was some variation of temperature and other conditions between them, the time scale for each batch was adjusted so that the period of minimum end-plate activity centred about a common mean value, about 5 days after nerve section (typical for a frog's sartorius at 20° C). The ordinate represents the percentage of impaled muscle fibres in which spontaneous miniature potentials were detected. In the normal control muscles (not shown in Text-fig. 8) this value varied, between 46 and 100%, with an average of 89%. That is to say, by using the microscopic procedure described on p. 146 in the normal muscle, nearly 90 out of every 100 electrode insertions revealed miniature e.p.p.'s. The open circles represent end-plates which still responded to nerve stimulation (and whose spontaneous activity was of normal rate and amplitude distribution); the filled circles



Text-fig. 8. The transient cessation of spontaneous activity after nerve section; pooled results from 46 frogs. Abscissa, time in days after operation (scaled as described in text); ordinate, percentage of impaled end-plates at which spontaneous activity was observed. Open circles, end-plates with transmission still functioning, spontaneous activity of normal frequency range and amplitude distribution; filled circles, end-plates at which no response to nerve stimulation was obtained; spontaneous activity was either absent, or of very low frequency, and of the abnormal amplitude distribution shown in Text-fig. 4.

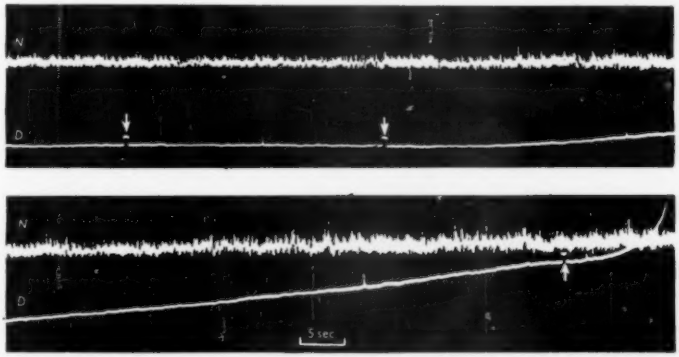
represent end-plates at which transmission had failed (and any observed spontaneous activity had the characteristic features of denervated muscle). It is clear from this figure that there was a silent period in the spontaneous activity, about 4–6 days after nerve section, followed by gradual resumption.

Observations after long-term denervation. As far as we could ascertain, the state of low-rate activity, once resumed, was maintained throughout the period of denervation. The observations became difficult after about 2 months, because the intramuscular nerves were no longer visible and the selection of 'end-plate regions' had to proceed by trial and error. Under these conditions the percentage of 'successful' electrode insertions inevitably dropped and could no longer be used as an index figure. Nevertheless, even after 130 days typical spontaneous miniature potentials were observed, and subsequent examination with the electron microscope confirmed the presence of denervated

end-plates in the selected region of the muscle. When no special precautions had been taken (see Methods), nerve regeneration intervened after 1–2 months and made itself evident, as already mentioned, by the appearance of a normal type of spontaneous miniature discharges even before impulse transmission was re-established.

Acetylcholine sensitivity and miniature potentials

It is well known that some weeks after denervation the sensitivity of frog muscle to applied acetylcholine increases greatly (Brown, 1937; Kuffler, 1943; Nicholls, 1956). The matter has been studied in detail and is reported elsewhere by Miledi (1959). There is wide variation in the time of onset and the degree of supersensitivity. It is sufficient here to mention two relevant findings. (i) The disappearance and subsequent resumption of spontaneous activity during the first 2 weeks are quite unrelated to the chemical sensitivity



Text-fig. 9. Spontaneous activity at normal (*N*) and 66-day denervated (*D*) end-plate. At the first arrow fresh Ringer's solution was very slowly run through the bath. At the second arrow the solution was switched to ACh-chloride (10^{-9} (w/v)); this led to progressive depolarization, and eventually spikes and twitching, in the denervated muscle. (At the third arrow, Ringer's solution was turned on again, but too late to arrest the ACh effect). Amplitude of pulse signal (at arrows): 1 mV for *D*, and 1·1 mV for *N*.

of the muscle; when tested at this stage approximately the same doses of ACh were required to set up twitches in denervated and control muscles, and there was no significant difference in the local depolarization produced by subthreshold doses. (ii) After a few months' denervation, when in some muscles the sensitivity to diffusely applied ACh had increased more than a hundredfold, there was no corresponding increase in the amplitude of the spontaneous potentials (cf. Text-fig. 9); it was doubtful whether there was any difference in size which could not be explained simply by the greater resistance of the somewhat atrophic denervated muscle fibres (Katz & Thesleff, 1957; Miledi, 1959). As has been pointed out by Axelsson & Thesleff (1959) and Miledi (1959), the

development of drug supersensitivity may be due largely to a spatial spreading of the chemo-receptive properties from the localized end-plate region to the rest of the fibre surface; in this case it would be observed only if the drug is applied to an area which exceeds the original site of the end-plate receptors.

In some experiments when the sensitivity to ACh was tested iontophoretically with a micropipette, intra- and extracellular applications to the muscle fibre were compared. The finding of del Castillo & Katz (1955), that the depolarizing effect of ACh is only observed during extracellular application, was confirmed in denervated muscles (several weeks after nerve section), which agreed with an earlier unpublished observation made in collaboration with Dr Thesleff.

In the present series of experiments spontaneous fibrillation was not observed. Unlike mammalian muscle fibres, where spontaneous twitching is a common phenomenon, denervated frog muscle has been found to fibrillate only infrequently, and then after very long periods of nerve section (Reid, 1941; Kuffler, 1943; Fatt & Katz, 1952).

DISCUSSION

In spite of several quantitative differences, a strong argument can be put forward for the view that the spontaneous activity at denervated end-plates is a renewal of substantially the same process as that occurring at normal end-plates, i.e. a release of packets of acetylcholine, or of a similarly effective substance, from a nearby source, on the outside of the muscle fibre. The argument rests on (i) the effects of curare and neostigmine, which act on the miniature potentials in the same way as on ACh-produced depolarizations; (ii) the fact that the potentials are discrete and sometimes fairly large, indicating that large numbers of molecules must impinge simultaneously on the end-plate; (iii) the rapid time course of many potentials, which suggests that the source of release is within close range of the receptors; and (iv) the fact that, as in normal muscle, intracellular application of ACh is ineffective.

There is ample evidence that normally the packets of ACh are stored in and released from the motor nerve endings. Where does the renewed activity originate? Before one can attempt to answer this question, it is necessary to find out what is the fate of the synaptic, and especially the presynaptic, structures in the course of nerve degeneration.

The obvious question arises whether the renewed activity is related to the spontaneous twitching which is commonly observed in denervated mammalian muscle fibres. This remains to be studied experimentally; but on the existing evidence fibrillation, unlike the miniature potentials or the effect of applied ACh, is not suppressed by curare (Langley & Kato, 1915; Reid, 1942), and the suggested relation seems therefore unlikely. In the frog fibrillation is an

unusual phenomenon and, on the few occasions when it has been reported, it was also found to be unaffected by curare (Reid, 1941; Fatt & Katz, 1952) which sets it apart from the phenomenon described in this paper.

In sympathetic ganglia, it has been shown that ACh synthesis vanishes within a short time after preganglionic nerve section (Feldberg, 1943; Banister & Scrase, 1950; see also Brown & Feldberg, 1936; MacIntosh, 1938). This does not necessarily conflict with our interpretation; for it would be impossible to say whether the production of ACh is completely abolished or persists at a level of less than 1% of the initial rate, and no more than this would be required to maintain the renewed end-plate activity.

If one considers the different frequencies of the discharge in normal and denervated muscle, the 'resting' (i.e. 'unstimulated') values hardly provide a fair index of the relative rates of production or of the ACh stores in the two tissues. Normally there is a very wide margin (approximately 10^6 times) between the spontaneous and the maximum rates of ACh delivery of which the nerve endings are capable. The limitation in discharge rate appears to reside in the mechanism of surface release, and not in the internal storage or synthesizing capacity of the axon terminals. At the denervated end-plate most of the ordinary means of stimulation (chemical, mechanical) failed to accelerate the background discharge; only by the use of hypotonic solutions was the frequency increased to about 1/sec. It may be that the maximum observable frequencies are more nearly representative of the available stores of ACh; if this be true, then the discrepancy between normal and denervated situations would be much larger than indicated by the 'resting' rates.

In more concrete terms the situation could be envisaged as follows. Normally there is a large turnover of ACh inside the nerve terminal, quantal packets of ACh being built up (perhaps in the form of vesicles) in large numbers and maintained in a steady average state. The growth of each individual parcel is accomplished quickly and occupies only a brief period in its life span which is terminated by destruction or release of its contents from the cell surface.

At the denervated end-plate a similar process goes on within an as yet unspecified structure on the outside of the muscle fibre. But there is an important quantitative difference: the production and growth of parcels of ACh proceeds at an extremely low rate compared with the turnover in normal nerve endings, so that in the steady state the population is of much lower density and also of much wider size distribution than normally. In normal muscle there is little chance of ACh quanta being released that have not already reached their steady-state 'adult' size. Conversely, in denervated muscle there is little chance of an ACh quantum reaching its adult size, for it is likely to be discharged at some random stage of its slow build-up period. An interesting feature of this hypothesis is that it could explain, not only the difference in rates of discharge between normal and denervated end-plates,

but the different types of size distribution of the miniature potentials. The 'normal' histogram is of the kind that would be expected when sampling an 'adult' population. The 'denervated' histogram would fit a population of growing individuals whose 'lives' terminate after random periods of linear growth.

PART II. Electron-microscope examination

The experiments described in the preceding part suggest that, in the immediate neighbourhood of the denervated end-plates, there is some source of a renewed, small-scale production of ACh, which persists for many weeks. To obtain further information the electrically explored end-plate regions of many denervated and control muscles were prepared after the experiment for electron-microscopic study.

METHODS

The procedure was the same as for normal myoneural junctions and has been described by Birks, Huxley & Katz (1959). The only difference was that the selection of an end-plate region became more difficult after several weeks of denervation because the fine intramuscular nerve branches were then no longer visible even after osmic fixation, and the electro-physiological location of miniature potentials, which was somewhat coarse in these cases, had to serve as the only guide. Nevertheless, after some experience had been gained no great difficulty was encountered in finding end-plate structures even after long-term (up to 130 days) denervation.

RESULTS

The normal myoneural junction of the frog shows three distinct cellular layers (Birks, Huxley & Katz, 1960); a nucleated Schwann cell which covers one side of the axon terminal and separates it from the connective tissue fibrils, the nerve-ending with its mitochondria and accumulation of '500 Å vesicles', and the muscle fibre with its array of junctional folds. It is possible to obtain sections which do not show all three junctional components, but such instances are rare and only apply to a small portion of any one junction.

After motor nerve section, three successive stages can be distinguished on our physiological evidence (see Text-fig. 8); a latent period of about 3 days during which normal transmission occurs at most of the junctions, a transient silent period when all junctional activity appears to have vanished, and a period of resumed low-rate spontaneous activity which persists for many weeks. The time course of these progressive changes varies with temperature, and for this reason alone it is necessary to examine the physiological state of each preparation if one wishes to look for correlated structural changes. It is not sufficient to record simply the mechanical response to a nerve stimulus, for in a muscle like the sartorius, whose fibres have a multiple nerve supply, a strong indirect twitch may be obtained at a time when the majority of the nerve endings have already ceased to function.

During the first stage (latent period), no significant structural changes were

observed. It should be pointed out that we were looking for obvious changes, i.e. a striking departure from the normal picture which has been described. Such changes were seen as early as 3–4 days after denervation in every muscle in which a wide-spread failure of transmission and spontaneous end-plate activity had occurred. During this phase a variety of gross alterations were seen in the nerve terminals, the full description of which is deferred until later. The abnormal features observed during this transitional stage were of a rather mixed and complicated kind; enlarged mitochondria whose internal structure was either in fragments or filled with unusually dense material, some nerve endings containing large masses of apparently agglutinated vesicles while others were almost empty and devoid of vesicles. The relation between Schwann cell and nerve ending was also highly variable and in many places quite obscure.

During the third, final, stage the electron micrographs were far simpler and easier to interpret. Instead of the normal triple-cell complex (Schwann cell, nerve terminal, muscle fibre), there remained now only two cells which were in close contact—a nucleated cell which had the appearance of the normal Schwann overlay and the muscle fibre with apparently normal junctional folds (Pls. 1–4). This general picture was found consistently throughout the period of denervation which we studied (up to 130 days provided re-entry of the nerve had been prevented).

The finding that the post-synaptic folds do not undergo any drastic change was of considerable practical use, for it provided us with one definite criterion in locating end-plates in denervated muscle. Our case for identifying the extraneous cell as a 'Schwann cell' is perhaps less secure; for the structural details alone are not sufficiently distinctive to differentiate it from other extraneous cells (fibroblasts, endothelial cells) in the neighbourhood. It is certainly not a remnant of the motor nerve ending; this possibility is ruled out by the presence of a nucleus (Pls. 2*B*, 3*B* and 4). Because of the close spatial relation of this cell to the junctional folds, we presume that it is identical with, or originates from, the terminal Schwann envelope, having absorbed and replaced the remnants of the nerve ending.

Plates 1–4 illustrate the final state in transverse and longitudinal sections; for comparison, normal transverse micrographs are also shown. The consistent difference between normal and denervated end-plates is that there is only a single 'presynaptic' cell after denervation. Its contact with the surface of the muscle fibre is indistinguishable from the normal synaptic contact between nerve and muscle: size and appearance of the synaptic space with its intermediate dense layer are much the same. But the cell contents resemble those of the Schwann cell and are quite different from those of a normal axon terminal. This applies not only to the presence of a nucleus, but to the variety of structural detail found in different regions of the cytoplasm. The rather

regular disposition of mitochondrial and vesicular zones, typical of nerve endings, is not seen, and the characteristic concentrations of '500 Å vesicles' are absent. Instead, one finds some regions of little structural detail showing only scattered fine filaments and granules, while other regions, situated frequently in the neighbourhood of the nucleus, show numerous particles, mitochondria as well as spherical granules and vesicles of widely varying sizes.

In Pl. 4 a longitudinal series of pictures at low magnification is shown from a 10-days-denervated end-plate, covering a strip over 50μ long. The section has passed through an extensive array of post-junctional folds which are covered from end to end and separated from the external collagen layer by a long nucleated cell, presumably the Schwann cell. This kind of observation is of interest because it illustrates the extent of the synaptic contact with the muscle fibre which the Schwann cell has established at this stage.

This situation appears to be maintained for many weeks, in spite of gross degenerative changes (atrophy, increased density of connective tissue) which take place in the muscle during prolonged terms of denervation. In some long-term preparations, we have seen regions in which the junctional folds had an unusually wide lumen filled with finely granular material, and also regions in which the Schwann cell was separated by collagen fibrils from the muscle folds. However, this was not a consistent finding, and it seemed more significant that even after the longest period of denervation (130 days), junctional regions could be found whose folds had a normal appearance, and whose contact relation, between Schwann cell and muscle fibre, was substantially the same as that observed after 2 weeks denervation. It seems safe to conclude that during the 'final stage' of denervation, when low-rate spontaneous discharge of miniature potentials has been resumed, the axon terminals have completely disintegrated and been replaced in their synaptic position by the Schwann cell.

When regeneration of the nerve had not been prevented by a further operation, re-entry of axons and formation of new synaptic terminals was observed as early as 4–5 weeks after the nerve section. In some of the new junctions the nerve endings were smaller than those normally seen and were covered by a disproportionately big Schwann overlay. The contents of the axon terminals were typical of normal endings and usually very rich in vesicles. Structural regeneration of nerve terminals was found in some preparations even though impulse transmission had not been restored (though a possible failure as a result of dissection or other damage could not be entirely ruled out); but even in these preparations there was evidence that the spontaneous activity at some end-plates had returned to the normal pattern of sizes and frequencies.

The transient stage. The intermediate phase, associated with sudden failure of transmission and cessation of spontaneous activity, was of interest principally because one might hope to obtain some clues as to the function of specific

cell components, e.g. the vesicles. Unfortunately, the earliest structural changes which we observed were too complex and involved too many aspects of the presynaptic elements to lead to any such definite conclusion. It was, however, encouraging to find that there were striking structural abnormalities in every preparation in which junctional activity had ceased. In 'mixed' muscles which contained inactive as well as functioning end-plates, a mixture of normal and degenerating nerve endings was found. We could not identify individual end-plates, but in one preparation there was a clear distinction between three junctional regions, two of which were inactive and showed gross structural changes, while the third contained a high proportion of junctions of normal activity and structure.

Among the various disintegrative changes in the nerve terminals the most outstanding feature is the appearance of large 'honeycomb' structures which look like masses of agglutinated vesicles (i.e. closely packed vesicles whose interstices are denser than their central cores, e.g. Pls. 5–8). Built into the honeycombs one finds numerous swollen mitochondria whose internal structure may be broken up or filled with dense material. At other end-plates, presumably in a somewhat more advanced stage, islets of such honeycombs are seen completely enclosed in the Schwann cytoplasm which has established synaptic contact with the muscle fibre. In other areas, sometimes of the same end-plate, a very different form of degeneration is seen, the nerve terminal appearing as a large, almost empty space, containing a few scattered vesicles and swollen mitochondria.

Many questions are raised by these pictures which it is not possible, at present, to answer. We do not know whether the different structural abnormalities (honeycombs, empty spaces) represent consecutive steps or different pathways of a degenerative process. The only definite statement that can be made is that they all represent some form of transition from the normal, triple-cell, to the final, double-cell compound of the junction.

We inferred from a day-to-day comparison of the functional and structural properties that the early changes in both occurred abruptly and on the same day. An attempt was made to improve the timing of this comparison, by using denervated 'cold-frog' preparations in the way described on p. 148, but the result was inconclusive. At the end of such experiments, many of the previously transmitting junctions had failed and their spontaneous activity, after a transient large increase, had fallen to a very low rate. In the electron microscope most of these junctions showed abnormalities, notably swollen mitochondria and a general reduction in the number of vesicles, but in view of earlier observations (Birks *et al.* 1960) it is doubtful whether these alterations are specifically related to the degenerative process or result from damage and presynaptic depolarization.

DISCUSSION

The general conclusion from these experiments is that motor nerve section is followed, after a delay of some days, by structural disintegration of the axon terminals together with failure of transmission and stoppage of the spontaneous end-plate activity. Within the following week spontaneous discharge is resumed at a low rate, and by this time the place of the nerve ending is found to have been occupied by the Schwann cell. An obvious, though not inevitable, inference is that the source of the renewed release and production of ACh (or similarly-acting substance) is inside the Schwann cell.

There seem to be few reports in the literature which have a direct bearing on these conclusions. Statements concerning the time of neuromuscular failure and of histological break-down of the nerve endings in the frog vary widely: between a few days, according to Cipollone (1897), and several weeks according to Sokolow (1874), intermediate values being given by Langley (1909). These discrepancies might have been largely accounted for by climatic differences between the frog cellars of Rome, St Petersburg and Cambridge; unfortunately the temperatures at which the denervated frogs were kept are not stated in these papers, and this would have a very large effect on the time course of degeneration (see p. 147; cf. also Bethe, 1903; Parker, 1933).

A very relevant finding was the observation by Tiegs (1932) on lizard muscle, that the 'cords of Büngner', which consist of Schwann cells and replace the peripheral portion of a cut nerve, make immediate contact with the muscle end-plates and remain in this position for long periods (tested up to 146 days).

An obvious suggestion is that there is a connexion between the terminal Schwann cell and the resumed activity at the denervated end-plate, because the physiological evidence indicates that there is a renewed, low-rate discharge of packets of ACh from a nearby source on the outside of the muscle fibre (see Discussion, p. 158), and the electron microscope shows a Schwann cell in the required place and throughout the appropriate period. There are, however, other possibilities which we cannot eliminate. For example, ACh might be released from the muscle fibre itself; this is not ruled out by the finding that iontophoretic release of the drug into the interior of the muscle fibre is ineffective. It is also possible that remnants of the axonal stock of ACh are lingering on or that there may have been 'sprouting' from nearby autonomic nerves, though one would then have to assume that the associated structures not only survived or escaped degeneration, but remained undetected in the electron microscope. These are unlikely suggestions, but the lack of any positive evidence does not, of course, disprove them.

If we accept the more probable view that the low-rate activity originates within the Schwann cell, a number of further intriguing questions arise. Are

these cells always capable of producing ACh, at a very low rate, or is this a new biochemical property which the cell acquires after absorbing the remnants of the nerve endings and of their enzymic apparatus? And in this connexion, how are we to explain the temporal gap between normal and resumed end-plate activities? This silent period would be fairly easy to understand if the production of an ACh-like substance by the Schwann cell were a new property which takes some time to develop. The alternative argument, that it would take time in any case for the cell to establish extensive contact with the muscle fibre, is not very strong, because during the silent period many points can be found at which the Schwann cell has already replaced the axon terminal in its synaptic position, and some areas of contact between Schwann cell and muscle end-plate, though much more restricted, exist even normally.

One may ask whether the view presented here is still compatible with the 'vesicular hypothesis', according to which quantal delivery of ACh depends on its distribution in discrete vesicular bags inside the nerve ending. There would seem to be no difficulty in extending this view to the denervated junction, for vesicles can certainly be seen inside Schwann cells, though much more sparsely distributed and in a much greater variety of sizes. These features might indeed be related to the lower frequency and wider amplitude distribution of the miniature potentials.

SUMMARY

1. The functional and structural changes which occur at the myoneural junction of the frog after motor nerve section have been investigated by intracellular recording of the electrical end-plate activity and by subsequent examination of junctional regions in the electron microscope.

2. The time course of the degenerative process varies greatly with temperature. At 20° C junctional transmission in the frog's sartorius fails after approximately 5 days; at about the same time the spontaneous discharge of miniature end-plate potentials ceases.

3. During the following week local spontaneous activity is gradually resumed at the denervated end-plates, though at a much lower rate than in the control muscle and with characteristic differences in distribution of the amplitude of miniature potentials and in their response to various stimulating agents. The renewed end-plate discharge is maintained at a low rate for many weeks.

4. The transient stoppage and subsequent resumption of spontaneous activity are not related to any change in chemical sensitivity of the denervated muscle. During the early stages the effects of applied drugs (acetylcholine, curare and neostigmine) are not substantially different from those in normal muscle. Supersensitivity to acetylcholine develops after some weeks of

denervation, when the spontaneous local activity has already been fully established.

5. Evidence is presented for the view that the miniature potentials at denervated end-plates arise, like those in normal muscle, from a quantal liberation of acetylcholine, or a similarly effective substance, from a nearby source on the outside of the muscle fibre.

6. Electron-microscopic examination shows that gross structural changes occur in the motor nerve terminals at the time of failure of transmission, sometimes as early as 3–4 days after nerve section. Among the prominent features are swelling and disintegration of mitochondria, and formation of large 'honeycomb' structures consisting of apparently agglutinated vesicles.

7. The final state is reached after about 1 week and is maintained throughout the period of denervation (which was followed up to 130 days). It is characterized by complete disappearance of the axon terminal and the presence of two, instead of the normal compound of three, cells at the end-plate: namely, the nucleated Schwann cell in contact with the muscle fibre whose junctional folds have retained their normal appearance.

8. It is suggested that the Schwann cell, which has replaced the motor nerve ending in its synaptic position, is responsible for a renewed, small-scale production and release of 'acetylcholine packets'.

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EXPLANATION OF PLATES

All electron micrographs are from frog's sartorius muscles, fixed in buffered OsO_4 and then stained with 1% phospho-tungstic acid in absolute alcohol. All scales = 1 μ.

Plate 1. Cross-sections, same magnification for *A*, *B* and *C*. *A*, normal, innervated, muscle, showing Schwann cell overlay, vesiculated nerve ending and muscle fibre with portions of junctional folds; *B* and *C*, 11 days denervation.

Plate 2. Cross-sections through nucleated regions of Schwann cell. *A*, normal muscle; *B*, 11 days after denervation.

Plate 3. Longitudinal sections of denervated junctions (*A*, 12 days; *B*, 10 days) showing variety of structural inclusions in certain regions of the Schwann cell. Nucleus is seen in *B*.

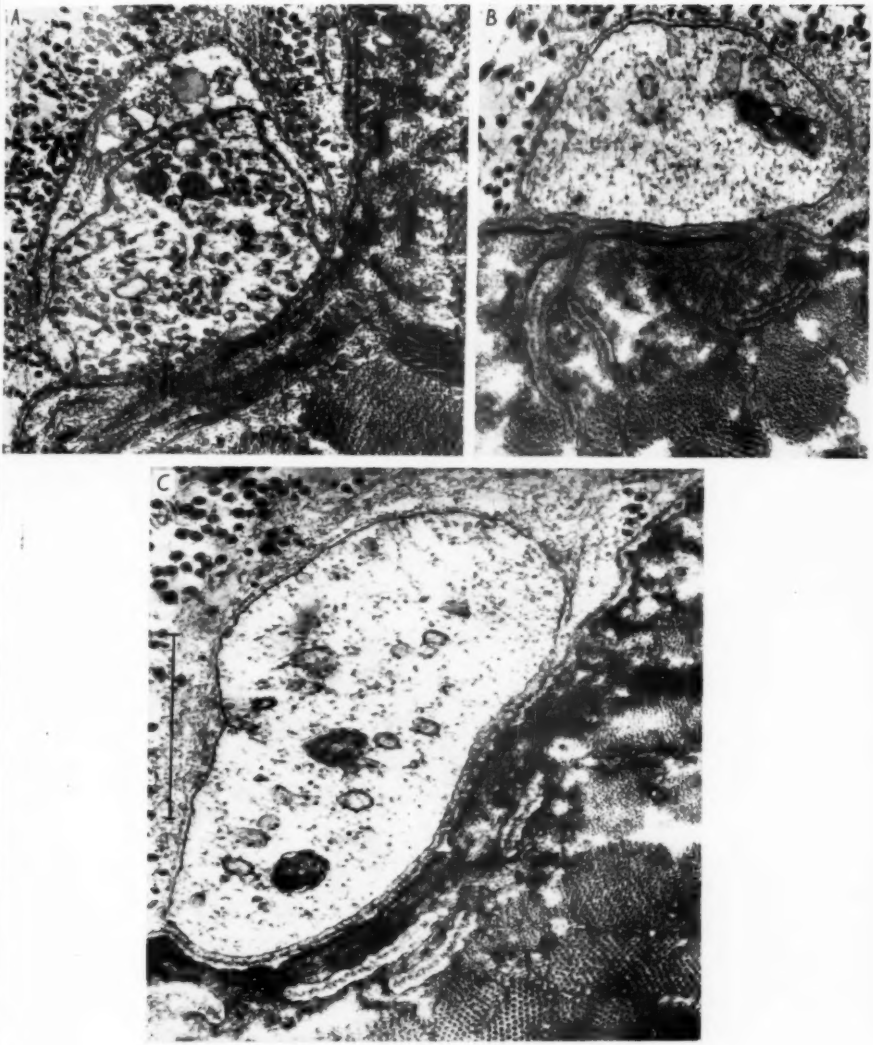
Plate 4. Longitudinal survey of a denervated junction of the muscle fibre (10 days). The surface of the muscle fibre with its junctional folds can be followed, from left to right and in successive rows from above, over a distance of more than 50 μ. It is covered from end to end by a 'Schwann cell' whose nucleus is seen in the second and third rows.

Plate 5. Early stage of denervation (4 days). Longitudinal section showing a dense, 'honeycombed', remnant of axon terminal surrounded by lightly stained cytoplasm (presumably Schwann cell). Muscle with junctional folds on the right.

Plate 6. Different forms of 'honeycombs'. *A*: transverse, 8 days denervation. *B*: longitudinal, 4 days denervation.

Plate 7. Two more examples of transient forms of nerve remnants, surrounded by Schwann (?) cytoplasm. 4-days denervation; longitudinal; same magnifications in *A* and *B*.

Plate 8. In this figure an attempt has been made to illustrate a sequence of the earliest morphological changes after nerve section; longitudinal, all of same magnification. *A*, 3-days denervation; transmission in this muscle was intact, and there were no obvious structural abnormalities. *B–E*, from a 'mixed' muscle (4 days); transmission had failed at many junctions, but was still functioning at some places. *B* and *C*, these were from a region at which normal and abnormal endings could be seen side by side; *B* and *C* probably represent one of the earliest structural changes (swelling of axonal mitochondria, low density of vesicles). *D* and *E*, regions of more advanced degeneration similar to those in Plates 5–7.



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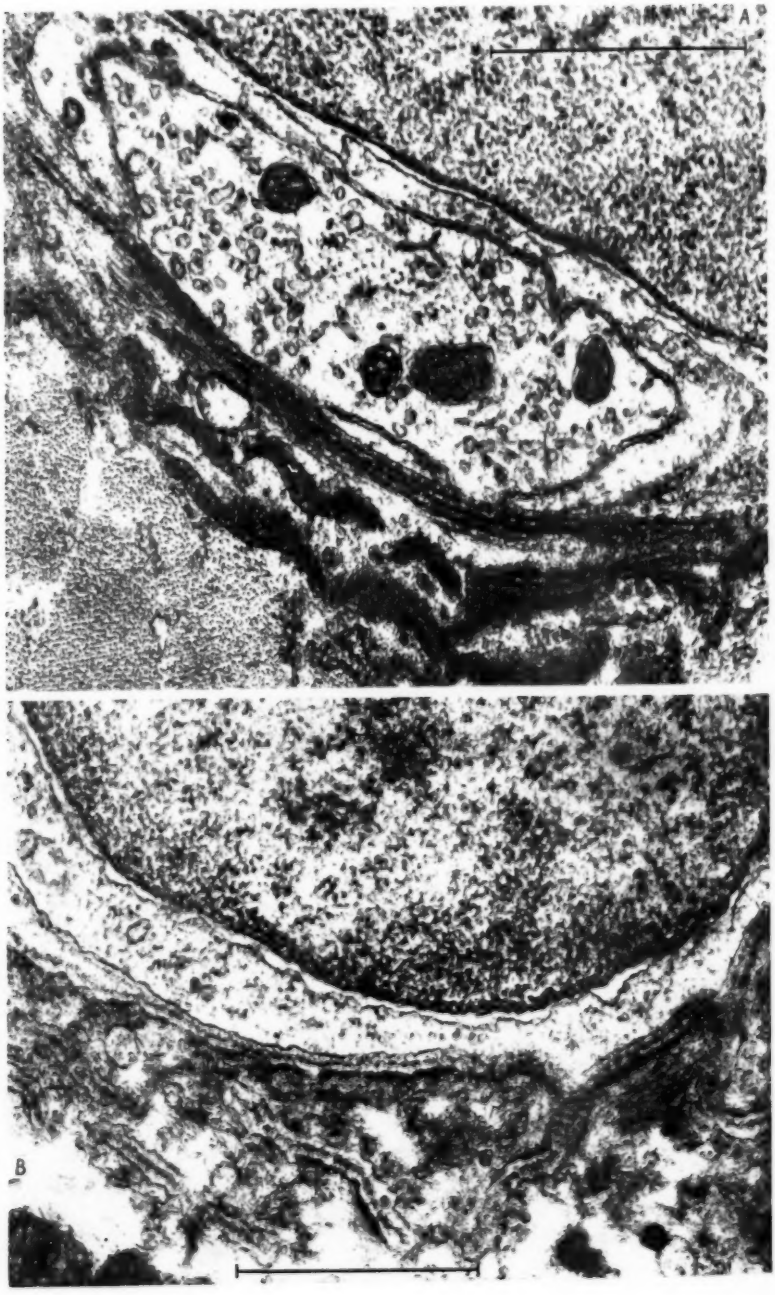
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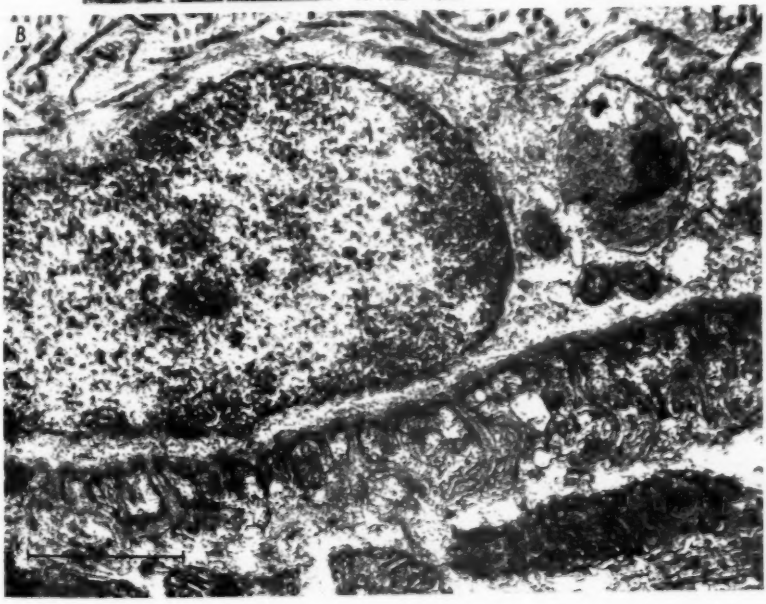
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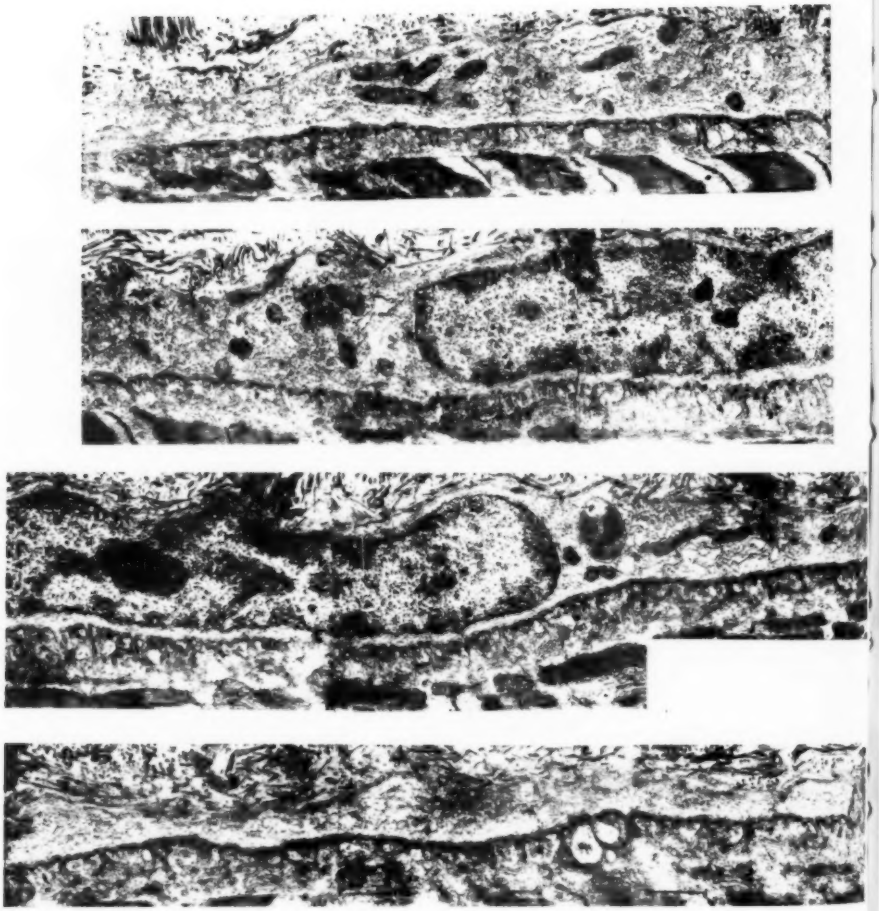

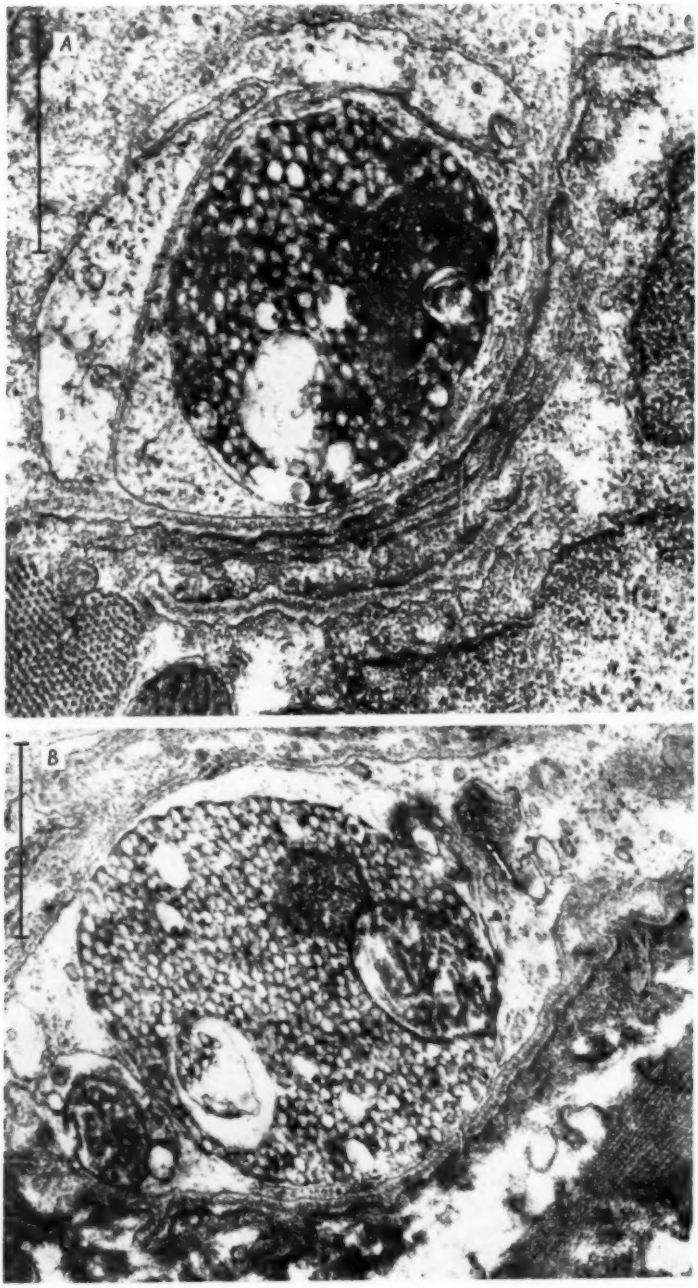
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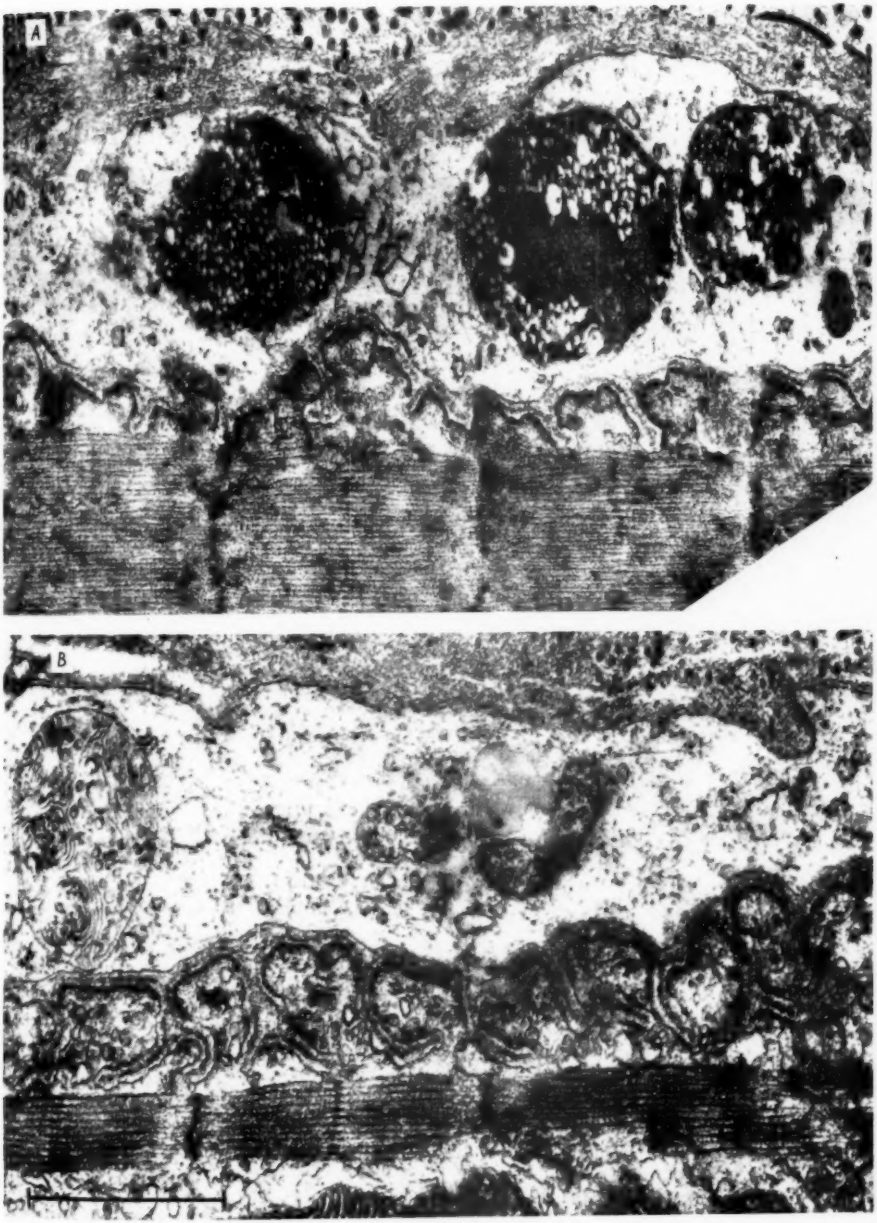
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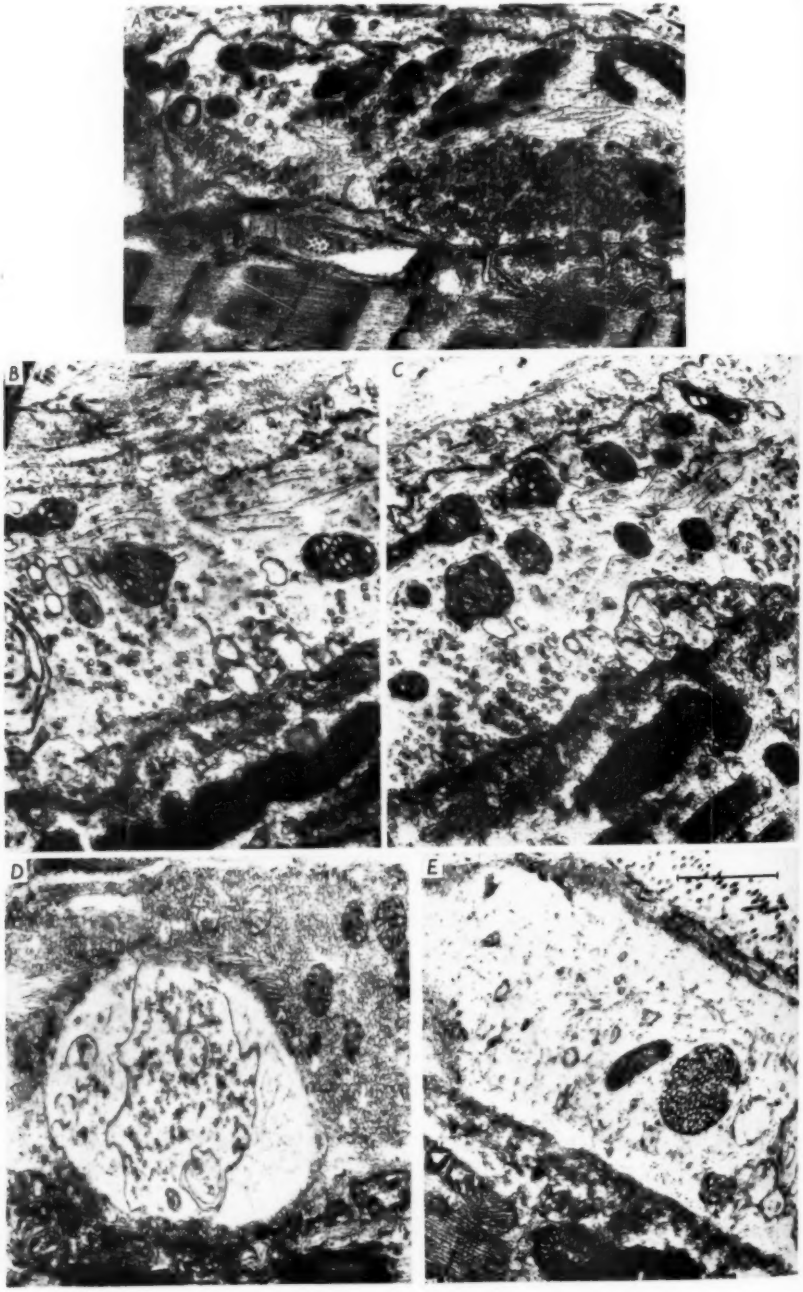
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THE ELECTRICAL ACTIVITY OF MAMMALIAN INTRAFUSAL FIBRES

BY C. EYZAGUIRRE

From the Department of Physiology, University of Utah College of Medicine, Salt Lake City, Utah, U.S.A.

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The function of mammalian spindles has been extensively investigated in recent years. From these studies it is apparent that stimulation of small-diameter ventral root axons increases the discharge rate of spindle afferent fibres. (The motor fibres to the spindle have been variously designated as gamma efferents (Leksell, 1945), small-nerve motor fibres (Kuffler, Hunt & Quilliam, 1951) and fusimotor neurones (Hunt & Paintal, 1958).) The available experimental evidence indicates that the increased sensory discharge during fusimotor activation results from contraction of intrafusal fibres. In fact, Kuffler *et al.* (1951) succeeded in recording a small potential from the muscle surface during selective stimulation of fusimotor neurones. This potential was localized in a small muscle area and these authors concluded that it must have originated from intrafusal muscle fibre activity. However, it could not be decided whether this 'intrafusal potential' resulted from action localized to the intrafusal neuromuscular junction or from activity propagated along the intrafusal fibres.

Activity of muscle fibres of qualitatively different types elicits, in lower vertebrates, either propagated or junctional potentials only. Thus, a 'twitch' and a 'slow' muscle systems have been established in the frog (Kuffler & Vaughan Williams, 1953*a*, *b*). Since mammalian extrafusal fibres are equivalent to the frog's 'twitch' fibres it was of interest to determine whether intrafusal bundles behave like those of the 'slow' system of the frog. It has long been suspected that mammalian intrafusal fibre contraction is slower than that of extrafusal elements, a deduction based upon the sensory discharge pattern evoked during stimulation of fusimotor neurones (Kuffler & Hunt, 1950). Results of the present study suggest that intrafusal fibre activity elicits propagated action potentials during fusimotor stimulation; consequently, intra- and extrafusal fibres are similar. A preliminary report has already been published (Eyzaguirre, 1959).

METHODS

Cats were anaesthetized with sodium pentobarbital. The tenuissimus muscle was excised, together with its own nerve and part of the sciatic (for details see Adrian, 1925). The preparation was mounted in a transparent chamber containing Locke's solution (NaCl 9·0, KCl 0·42, $CaCl_2$ 0·24, and $NaHCO_3$ 0·2 g/l.); dextrose 1·0 g/l. was added immediately before the experiment. A gas mixture containing 95 % O_2 and 5 % CO_2 was bubbled into the saline, which was covered by a layer of paraffin oil. The temperature of the bath was maintained at 37–38° C by means of an insulated nichron loop connected to a battery. Both ends of the muscle were firmly gripped by forceps which were part of a 'stretcher' operated by micrometer screws; this device allowed careful adjustment of the resting tension. In general, the experimental arrangement was similar to one used for cold-blooded preparations (Eyzaguirre & Kuffler, 1955; Eyzaguirre, 1957, 1958) except for the heating element. With the aid of a binocular dissecting microscope and polarized light the preparation was cleaned from connective tissue. The nerve was laid flat on a glass plate and split with sharp dissecting needles in order to isolate single motor and sensory fibres. If the nerve sheath proved too tough the nerve was placed in a solution of papaine or chymotrypsin (1 mg/ml.) and digestion was allowed to proceed for 30–60 min. After that time connective tissue was considerably softer and isolation of single fibres relatively easy. Isolation of fusimotor fibres proved somewhat difficult when the nerve was teased close to the muscle; it was easier to isolate them high up in the sciatic nerve. Normally, fusimotor fibres were identified by their effect upon stimulation on sensory discharge rate in the absence of extrafusal muscular contraction. In a number of experiments fusimotor fibres were stimulated by axon reflex; one nerve branch to the tenuissimus was cut close to the muscle and stimulating electrodes were placed on its proximal end. Nerve action potentials were recorded from small filaments which often contained single sensory fibres. Sometimes it was possible to detect visually a slender filament contracting during fusimotor stimulation. Contraction of such a filament coincided with increased sensory discharges. Intrafusal potentials were recorded from the surface of the muscle immersed in paraffin oil by small (50 μ) platinum-iridium electrodes connected to capacity-coupled amplifiers. High-frequency filters were used in order to decrease amplifier noise. The filtering used did not appreciably change the amplitude or time course of most intrafusal potentials. If the potentials recorded were sufficient in amplitude for ease of measurement the fusimotor fibre was stimulated at 1/sec. However, when the signal: noise ratio was low, about 10 responses were superimposed at a stimulation frequency of 10/sec.

Several drugs were applied either to the whole muscle or locally. For topical application a portion of the muscle was lowered into a small box containing a solution of the substance, while the rest of it remained in oil. Thus, intrafusal potentials could be recorded during drug action. In other experiments 11 % sucrose was applied in similar fashion (Fig. 4*A*).

RESULTS

Location of spindles

With the muscle suspended in oil, a spindle which responded to stimulation of a given fusimotor fibre could be located by determining the longitudinal distribution of the resulting intrafusal potential. In some cases location was aided by seeing the contraction of an intrafusal bundle. If this was not possible one electrode was fixed at one end of the muscle while the other recorded the response to fusimotor stimulation at successive points 1 mm apart along the length of the muscle. At each point the response was

recorded photographically. From these data a map of the longitudinal distribution of the intrafusal potentials was constructed. In most instances intrafusal potentials were recorded with one electrode leading and the other indifferent. An intrafusal bundle is only a short and slender filament surrounded by an encapsulated lymphatic space as well as a mass of 'inert' muscle tissues. Consequently, conditions similar to those in a volume conductor were present in this situation, although the preparation was immersed in oil. In some cases it was clear that the leading electrode picked up the electric potentials of more than one intrafusal bundle (see later).

The amplitude, duration and polarity of the intrafusal potentials varied from one experiment to another. The usual sequence of potential change as the roving and leading electrode approached a spindle region was from a purely positive deflexion to a positive–negative deflexion. When the electrode was further advanced to the active region a positive–negative–positive deflexion was registered. When a positive–negative deflexion was obtained, this sequence of events indicated that such a region acted as a source and then as a sink for current flow and hence was apparently at the end of the spindle. As the electrode was advanced further, the positive–negative–positive potential probably indicated that the region acted as a source, then a sink and then a source for current flow and that presumably was at the centre of the spindle. In some instances negative–positive deflexions were obtained. The latter, however, were difficult to obtain and apparently were produced by superficially located spindles. In other cases only small positive deflexions were registered which did not change appreciably by shifting the recording electrode within an active locus. It is suspected that this type of potential originated from deep-lying spindles.

Figure 1 shows the results of a methodical exploration of a muscle 80 mm long. A single fusimotor fibre was stimulated at 10/sec while one indifferent recording electrode (*Li*) remained stationary at the right end of the muscle (point 80). The other leading electrode (*La*) was moved from point 80 to point 0 (left end of the muscle). Exploration from point 80 to point 45 did not show any intrafusal potentials. At point 45 a small positive deflexion appeared which did not change significantly up to point 35. Between points 33 and 31 a positive–negative deflexion was recorded. At point 31 a triphasic potential appeared and this response was well recorded up to point 29. After point 29 a mainly positive deflexion was recorded (see point 27). At point 26 and toward point 0 another complex appeared, partly superimposed on the previously recorded potential (not illustrated). This last complex apparently originated in another spindle innervated by the same fusimotor fibre. When *La* was kept stationary at any point between points 0 and 45, the recorded potential

did not change when *Li* was moved from point 45 toward the right end of the muscle (point 80). The shaded area in the illustration indicates the probable position and length of the spindle. In other experiments larger intrafusal potentials were recorded, but the sequence of potential changes was similar to that presented in Fig. 1. Figure 2 illustrates such an instance. A 65 mm muscle was explored in millimetre steps and a single fusimotor fibre was stimulated at 1/sec. When both recording electrodes

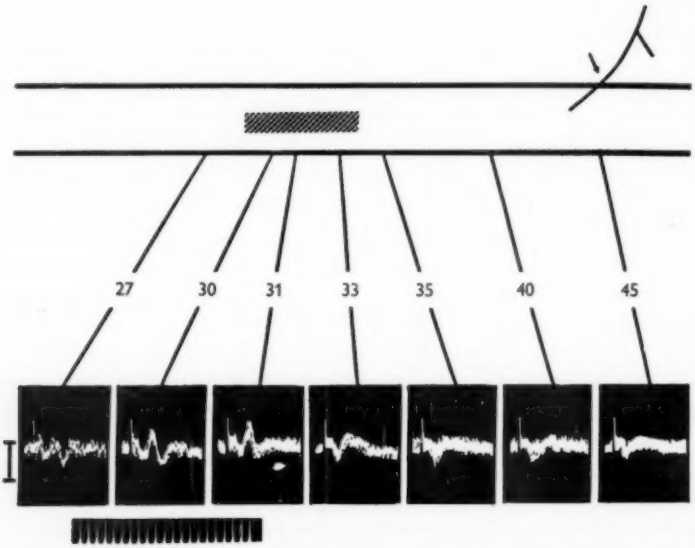

Fig. 1. Electrical exploration of tenuissimus muscle during stimulation of single fusimotor fibre at 10/sec. Muscle was 80 mm long. One electrode (*Li*) placed at an indifferent position (point 80) the other electrode (*La*) explored the surface in millimetre steps. Numbers indicate the position of *La* expressed in millimetres from left end of muscle (point 0). Arrow shows the point of nerve entry into the muscle. Voltage, 20 μV. In all figures except 8*A*, *B*, *D*, *E*, time marker 1 msec.

were placed at any points to the left of point 40 intrafusal potentials were not recorded. *Li* was left fixed at point 40 and *La* was moved past this point toward point 65 (right end of the muscle). At point 55 a small positive–negative deflexion occurred, the negative phase increasing in amplitude up to point 63 where a late positive deflexion appeared, which was more conspicuous at point 65. It is probable that the spindle in this case was located between points 55 and 65 (shaded area).

From these experiments it is concluded that electric exploration of the muscle surface during fusimotor activation allows the location of active intrafusal bundles provided that a single fusimotor fibre is stimulated.

Stimulation of more than one spindle efferent fibre produced intrafusal potentials which were too complex to permit accurate location of the spindle (see below). Further proof that electrical exploration is an adequate means for determining the position of a spindle was provided by experiments in which curare was applied locally. After electrical location of a spindle topical application of curare at this region completely blocked the appearance of the intrafusal potential (p. 179).

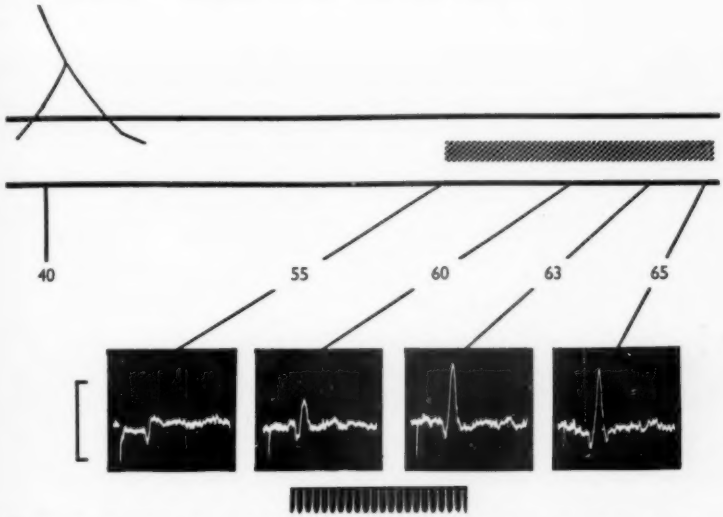

Fig. 2. Exploration of tenuissimus muscle during single fusimotor fibre stimulation at 1/sec. Muscle was 65 mm long. *Li* placed as an indifferent electrode at point 40. Numbers indicate the position of *La* on the surface (see text). Voltage, 20 μV.

The intrafusal potential

It was shown in the previous section that stimulation of a single fusimotor fibre elicited intrafusal potentials which were relatively simple and similar to potentials registered in a volume conductor (Figs. 1, 2). However, stimulation of more than one fusimotor fibre frequently produced complex polyphasic responses. Such potentials are illustrated in Fig. 3. The muscle was immersed in oil and the leading electrode was kept stationary at a given site. The indifferent electrode was placed 35 mm away on an electrically inactive region. At threshold stimulation a single fusimotor fibre was stimulated and the resulting intrafusal potential is shown in Fig. 3*A*. The intensity of stimulation was slowly raised and an additional intrafusal response appeared, partly superimposed on the previous complex (Fig. 3*B*). With stronger stimulating shocks a larger intrafusal potential

appeared near the end of the previous potentials (Fig. 3*C*). Results illustrated in Fig. 3*A*, *B* and *C* show that when several fusimotor fibres are stimulated the resulting intrafusal potential may become complicated, since unitary responses may appear close to and superimposed on each other. Also, the polarity of the potentials recorded by one electrode at a given site may show different characteristics. Thus, the potential illustrated in Fig. 3*A* was formed mainly by a positive deflexion, whereas the complex recorded in Fig. 3*C* was formed by the potentials illustrated in

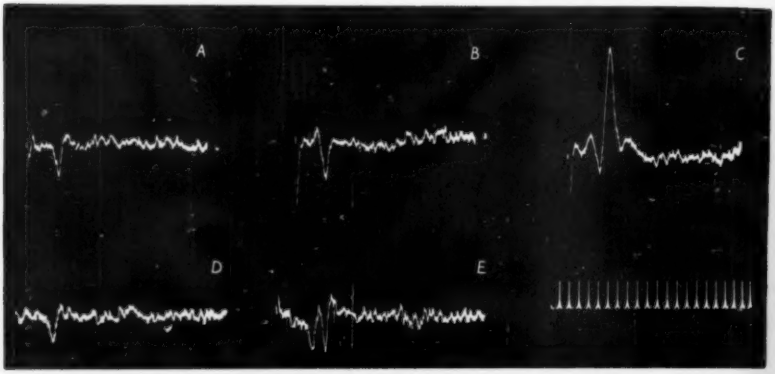

Fig. 3. Intrafusal potentials elicited by stimulation of several fusimotor fibres; leading electrode kept stationary at a given recording site, indifferent electrode 35 mm away. *A*, threshold stimulation evoked an intrafusal potential with prominent positive phase. *B*, activation of an additional fusimotor fibre elicited a smaller potential partly superimposed on previous one. *C*, a larger and mainly negative intrafusal potential produced by additional activation of a third fusimotor fibre. *D* and *E*, taken from different experiments: *D*, intrafusal potential produced by threshold fusimotor stimulation; *E*, additional potential was evoked by activating also another fibre.

Fig. 3*A* and *B* plus a large negative deflexion. In short, at a given recording site the leading electrode may record the sum of potentials of opposite polarity. Compounded intrafusal potentials appearing in close succession were not always the rule. At times, stimulation of more than one fusimotor fibre elicited intrafusal potentials separated by sufficiently long intervals to make them appear as separate entities. This occurrence is illustrated in Fig. 3*D*, *E*. Weak shocks delivered to a nerve filament activated a single fusimotor fibre, and the resulting intrafusal response is illustrated in Fig. 3*D*. Stronger fusimotor shocks produced an additional and slightly larger intrafusal potential after some delay (Fig. 3*E*).

The analysis of intrafusal potentials was difficult when both recording electrodes led off the intrafusal responses from the muscle surface and it

was not known which component of the intrafusal potential was being recorded by one or the other lead. This complication was sometimes unavoidable, even when a single fusimotor fibre was stimulated, since the spindles in the tenuissimus are placed serially (Boyd, 1956) and frequently a single fusimotor fibre may divide and innervate more than one spindle (Hunt & Kuffler, 1951). In order to eliminate this difficulty experiments were designed to produce a localized block under one electrode or between the recording leads. Figure 4 illustrates such a procedure. The muscle was lifted into oil and the spindles were located either by visual observation or by electrical exploration. That portion of the muscle was lowered into a small box and the recording leads were placed at both edges of the groove

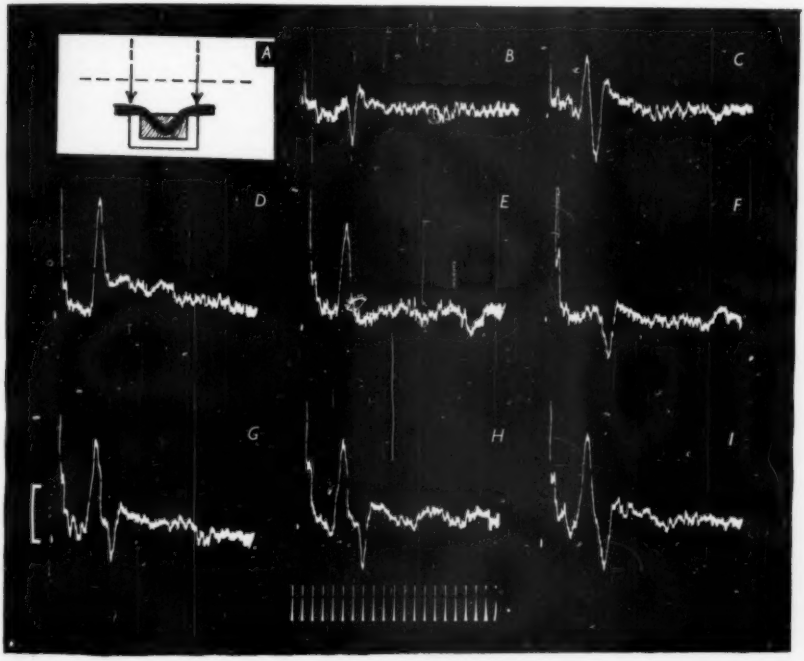

Fig. 4. Complex intrafusal potentials recorded from muscle surface, recording electrodes set 30 mm apart. Two fusimotor fibres stimulated by shocks of different intensities. *A*, schematic diagram of recording conditions (see text). *B*, threshold stimulation elicited one intrafusal potential. *C*, stronger shocks produced an additional intrafusal potential. Between *C* and *D* 11 % sucrose was applied for 10 min between recording electrodes; *D*, larger and monophasic intrafusal potential. *E*, 10 min after *D*. *F*, 10 min later weaker shocks again elicited intrafusal potential previously recorded in *B*. *G–I* illustrate further recovery from sucrose. Voltage, 16·5 μV.

(Fig. 4*A*). During control recording the box was filled with oil. In this particular experiment the recording leads were set 30 mm apart. Intensity of stimulation to the fusimotor fibres was gradually increased. At threshold stimulus strength an intrafusal potential averaging 13·2 μV was recorded. This potential had a shock–peak latency of 4·18 msec $\pm$ 0·07 S.D. (Fig. 4*B*). Stronger stimulation elicited an intrafusal potential of 29·7 μV measured from peak to peak. Shock–peak latency of the upward deflexion was 3·24 msec $\pm$ 0·08 S.D., while the downward deflexion had a latency of 4·17 msec $\pm$ 0·06 S.D. (Fig. 4*C*). It may be noticed that the shock–peak latency of the potential illustrated in Fig. 4*B* and that of the downward deflexion of the potential illustrated in Fig. 4*C* are the same. The following procedure was used to determine which lead recorded the potential in 4*B* and whether the complex illustrated in 4*C* was being recorded by the same lead or if its components resulted from bipolar leading: The groove in the box was filled with a solution of 11 % sucrose and the preparation remained otherwise undisturbed for 10 min. After this period of time the sucrose was removed and the intrafusal responses were recorded in oil as before. The recorded potential became monophasic and larger (32·3 μV) with a latency of 3·5 msec $\pm$ 0·4 S.D. In addition, a rather prominent negative after-potential was detected (Fig. 4*D*). The preparation remained undisturbed for another 10 min and the intrafusal potentials evoked again by fusimotor stimulation at different stimulus strength. Fusimotor stimulation produced *only* an upward deflexion averaging 24·7 μV, with a shock–peak latency of 3·3 msec $\pm$ 0·14 S.D. This is the same response registered earlier during the full sucrose effect although now it had lower amplitude (Fig. 4*E*). A few minutes later it was possible to elicit an intrafusal potential similar to that recorded in Fig. 4*B* by reducing the stimulus strength. Its amplitude was now 16·3 μV and its shock–peak latency was 4·9 msec $\pm$ 0·14 S.D., which is significantly longer than that obtained before sucrose. Slowing down of conduction was obviously produced by sucrose although the site of its action was not determined (Fig. 4*F*). Stronger stimulation now elicited a diphasic potential in which, after the passage of time, the latency of the downward deflexion became progressively shorter until it reached normal values (Fig. 4*G–I*).

It is concluded from the experiment just described that the upward deflexion of the potential illustrated in Fig. 4*C* was recorded by the electrode proximal to the block, since it was not eliminated by sucrose. The intrafusal potential illustrated in Fig. 4*B* and the downward deflexion recorded in 4*C* were registered by the distal lead since they were blocked by sucrose. With regard to the site of origin of these potentials, it is suggested that two intrafusal bundles were involved. One was outside and proximal to the sucrose block, its responses being recorded by the proximal

electrode. The other intrafusal bundle must have been at least partly inside the sucrose solution and its responses were picked up by the distal electrode. This assumption is based on the facts that at least 20 mm of muscle was immersed in sucrose and that the length of the spindles is from 4 to 12 mm (Boyd, 1956). Consequently, the possibility that the recorded intrafusal potentials all originated in one intrafusal bundle could be excluded.

It is of some interest that different intrafusal potentials showed in many instances appreciably different latencies. These potentials had occasionally the same polarity as those illustrated in Fig. 3*D* and *E*, or their polarity could be different (Fig. 3*A*, *B*, *C*; Fig. 4*B*, *C*). In view of the fact that the position of the leading electrode does not appreciably influence the shock–peak latency of an intrafusal potential, as was shown in the preceding section (cf. also Kuffler *et al.* 1951), it is suggested that different latencies could be due to the following factors: (i) potentials originating in different spindles serially located, the differences in latency being accounted for by longer intramuscular nerve distances and (ii) by differences in conduction velocities in motor fibres to the same spindle. Experiments in which a localized sucrose block was employed seem to indicate that the first of the outlined possibilities is more likely to occur.

In several experiments stimulation of a single fusimotor fibre elicited intrafusal potentials in widely separated areas of muscle: intrafusal potentials could be localized to both ends of the muscle while the centre of the preparation remained electrically silent. Also, activation of intrafusal bundles by motor axon reflex (see Methods) was possible. These observations show that a single fusimotor fibre may divide and innervate widely separated muscle regions. Conversely, stimulation of more than one fusimotor fibre produced at times intrafusal responses with the same latency (Eyzaguirre, 1960). This finding probably indicates that a single intrafusal bundle may receive more than one fusimotor fibre. These results agree with previous observations of Hunt & Kuffler (1951).

Recovery of the intrafusal potential

Kuffler *et al.* (1951) reported that the intrafusal potentials do not show facilitation during repeated excitation. Furthermore, they found that if the interval between two successive fusimotor shocks was shortened to about 1 msec, the second intrafusal response was reduced. These points were reinvestigated and somewhat more detailed information is presented in this section.

The intrafusal potentials were recorded during nerve stimulation by paired shocks separated by varying intervals. It was necessary to ensure that only one fusimotor fibre was present in the nerve filament set up for

stimulation; when more than one spindle motor fibre remained, closely spaced, supramaximal shocks could activate more than one fibre, resulting in larger intrafusal potentials. The cathode was placed toward the spindle in order to avoid anodal polarizing effects. As the interval between shocks was decreased to 0·7 msec the response to the second (test) stimulus disappeared in all-or-none fashion. Figure 5 shows amplitude values of the response to the test stimulus expressed as percentage of the potential obtained during the first (conditioning) stimulus. At 0·7 msec interval the response to the test shock appeared, having an amplitude which averaged 98 % of that produced by the conditioning shocks. The second response

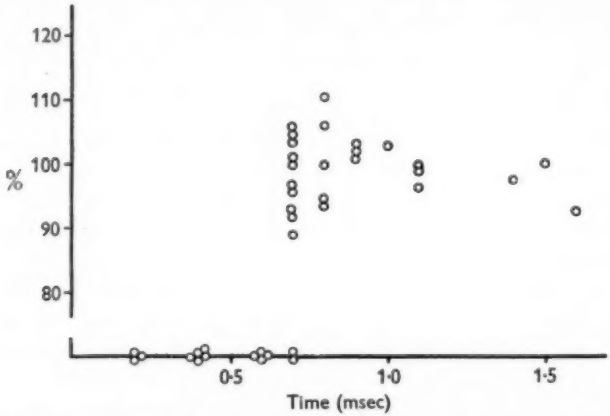

Fig. 5. Two nerve shocks delivered at different intervals. The diagram illustrates the amplitude of the second intrafusal response as compared with the first intrafusal potential. Abscissa, time; ordinate, amplitude of second intrafusal response expressed as percentage of the first potential.

failed to appear in 16·6 % of the cases and no intermediate steps were observed. Another interesting feature is illustrated in Fig. 6. When the intervals between stimuli were greater than 3·4 msec both potentials had the same shock–peak latencies, since all values recorded form a straight line with a slope of 1. At shorter intervals the onset of the second potential was delayed, since the points of the curve abandon the straight line for another with a gentler slope.

Results presented in Figs. 5 and 6 indicate that when two shocks to the fusimotor fibres are delivered in close succession the second intrafusal potential disappears abruptly; at longer intervals its amplitude does not differ significantly from the first one. The longer latency of the responses obtained after the test stimuli was probably due to longer neuromuscular delay caused by partially refractory intrafusal fibres. The latter situation

has been observed when dealing with extrafusal muscle fibres (Kuffler, 1942).

In general, the intrafusal neuromuscular junctions proved to be remarkably resistant to fatigue. When tetanic stimulation at 50–200/sec was maintained for a few seconds the amplitude of the intrafusal potentials did not change. However, if stimulation at these frequencies was maintained for a longer period of time a decline in amplitude of the intrafusal potentials was observed.

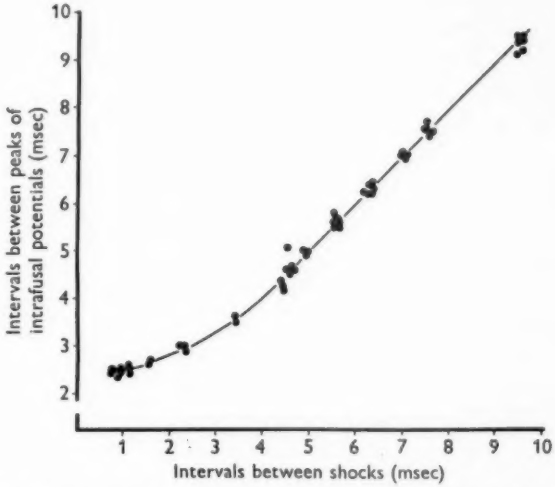

Fig. 6. Delayed onset of the second intrafusal response at short stimulating intervals; two stimulating pulses delivered at decreasing intervals.

Effect of curariform agents and physostigmine on intrafusal junctions

Amphibian intrafusal junctions are more resistant to fatigue and curarization than extrafusal ones. This property has been employed successfully in the study of spindle function without the interference of extrafusal effects (Katz, 1949; Eyzaguirre, 1957, 1958; Henatsch & Schulte, 1958). Experiments were designed to test whether this differential block could also be used in the mammalian preparation. On the whole these attempts were unsuccessful, since the margin between extra- and intrafusal neuromuscular block was too narrow to permit adequate working conditions. Extrafusal block was achieved by adding tubocurarine to the saline in concentrations of from 5 to 6×10^{-7} (w/v). Intrafusal junctions remained unimpaired only for several minutes after extrafusal block. Lower doses of this substance failed to produce block in both types of junctions. Similar results were obtained with Flaxedil (gallamine

triethiodide; American Cyanamid Co.) in concentrations of 3×10^{-6} (w/v), since several trials failed to reveal a clear selective action. Better differential action was obtained with Metubine (dimethyl-tubocurarine iodide; Lilly) in concentrations of from 3 to 5×10^{-7} (w/v). However, this drug had some effect on the intrafusal junctions at these concentrations, since at high frequency motor stimulation the sensory discharge rose rapidly to a peak and declined briefly afterwards while the preparation was still being stimulated. More troublesome was the fact that the differential action of Metubine was unpredictable. Thus, in some experiments a clear selective block was obtained, whereas in others results were doubtful. Larger doses of Metubine clearly blocked intrafusal junctions as well.

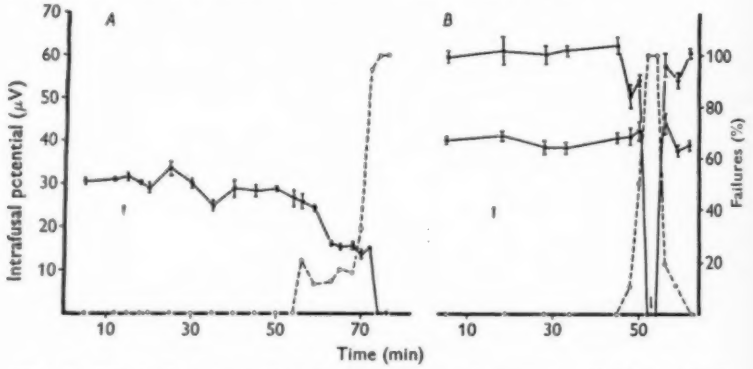

Fig. 7. Effect of curare on intrafusal junctions. *A*, curare applied to the bath ($1{\cdot}2 \times 10^{-6}$(w/v)) at arrow. Solid line (filled circles) shows the amplitude of the intrafusal potentials ± S.E.; broken line connects the points where the percentage of all-or-none failures of transmission was recorded (open circles). *B* illustrates an experiment in which two intrafusal potentials were simultaneously recorded; solid circles amplitude of intrafusal potentials ± S.E.; open circles percentage of all-or-none failures of transmission. Upward arrow, local application of curare 3×10^{-6} (w/v); downward arrow, preparation washed in fresh saline.

In order to test the manner by which curare blocks intrafusal neuromuscular transmission, spindles were located either by visual observation or by electrical exploration. Recording was accomplished in some experiments by keeping most of the muscle in saline while one end was lifted into the oil. Under this condition tubocurarine was added to the bath. However, in most instances the position of the spindle was such that local application was necessary (see Methods). Figure 7 *A* illustrates an instance in which tubocurarine $1{\cdot}2 \times 10^{-6}$ (w/v) was applied to the bath. The amplitude of the intrafusal response fluctuated around 28 μV with fusimotor stimulation at 1/sec. Several pictures were taken at each point in order to analyse the potential amplitude statistically. This was found

necessary because of base-line noise. During curarization potential amplitude did not change significantly for 49 min. After this period of time a sudden and significant drop in amplitude was observed. Potentials remained at this new level for 7 min but disappeared abruptly immediately afterwards (solid line). Washing the preparation in fresh saline restored transmission to normal levels (not illustrated). A second curve (broken line) shows the percentage of instances in which all-or-none failure of responses was recorded. A similar situation was present in another experiment in which curare was applied locally in concentrations of 3×10^{-6} (w/v) (Fig. 7*B*). Two distinct intrafusal potentials were recorded simultaneously. The larger one averaged about 60 μV while the other was of the order of 40 μV during fusimotor stimulation at 1/sec. Thirty-two minutes after the drug was applied (at upward arrow) the larger potential suffered a drop in amplitude while the smaller one did not. At this point both potentials failed to appear in 50 % of the cases. Four minutes later all intrafusal responses disappeared abruptly. After that period of time curare was replaced with fresh saline and the responses returned in 60 sec. The larger potential recovered to values intermediate between control responses and the first drop observed before its complete disappearance; the smaller recovered to normal values. The sensory discharges were controlled before and during application of curare in both experiments. Sensory acceleration during motor stimulation to the spindles disappeared during full curarization. It is evident from Fig. 7*A* and *B* that curare blocks intrafusal neuromuscular transmission either in stepwise or all-or-none fashion. Graded blocking effects have not been observed. This is true for any effective dose of the drug, since threshold curarizing doses of from 1 to 2×10^{-6} (w/v) produced similar effects.

In several experiments physostigmine salicylate was applied either to the whole bath or locally in concentrations of from 5×10^{-7} (w/v) to 3×10^{-8} (w/v). This substance did not change the amplitude or time course of the intrafusal potential. However, it occasionally produced intrafusal repetitive responses after single or paired nerve shocks. Figure 8*A–C* illustrates an experiment in which intrafusal repetitive responses were obtained after applying physostigmine 10^{-6} (w/v) for 35 min. Figure 8*D* and *E* shows the disappearance of repetitive responses once the stimulating frequency was increased to 10/sec. This observation means that when the intrafusal neuromuscular preparation is poisoned by the anticholinesterase, changes occur at the intrafusal neuromuscular junction which may fire the intrafusal fibres in an asynchronous manner.

Eccles, Katz & Kuffler (1942) observed repetitive spikes in extrafusal muscle fibres after injecting physostigmine. Part of this effect was due to nerve discharges caused by an action of the drug on the motor nerve

endings. The intrafusal repetitive responses observed in this study could have been produced by a prolonged transmitter action or by asynchronous fusimotor discharges during physostigmine application. No attempt was made to elucidate this point. However, intrafusal repetitive discharges looked as if the intrafusal fibres were firing asynchronously, judging by their shorter time course as compared to that of the evoked intrafusal potential. This fact would indicate that repetitive firing was due to a long-lasting transmitter action. An effect of physostigmine on the motor nerve terminals would, more likely, produce synchronous repetitive discharges.

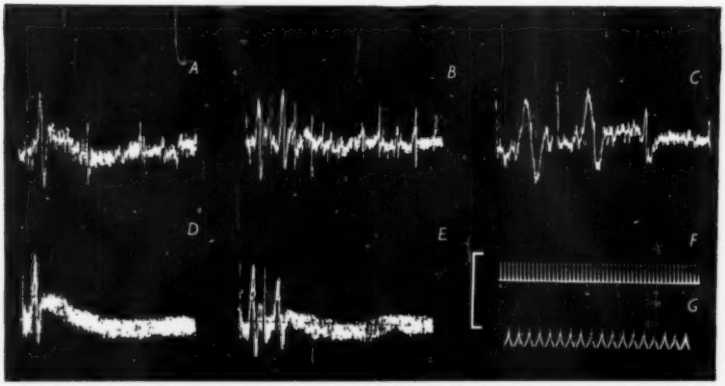

Fig. 8. Effect of physostigmine on intrafusal potentials. Before *A* physostigmine 10^{-6} (w/v) was applied locally for 35 min. *A*, repetitive responses evoked by single fusimotor shock. *B*, effects produced by two fusimotor shocks closely spaced. *C*, same as *B* but faster time base. *D* and *E*, blockage of repetitive responses during stimulation of the fusimotor fibre with one and two pulses respectively at a rate of 10/sec. *E*, voltage 20 μV; *F*, time marker—10 msec for *A*, *B*, *D* and *E*; *G*, 1 msec for *C*.

When intrafusal neuromuscular transmission was blocked by curare, application of physostigmine restored transmission in all-or-none fashion. In fact, in one experiment complete intrafusal neuromuscular block was obtained after local application of curare 10^{-6} (w/v). Physostigmine in concentrations of 10^{-6} (w/v) was then added and transmission was fully restored in 18 min. No transitional amplitude steps were observed in this process.

DISCUSSION

The main task of the present investigation was to learn whether the mammalian intrafusal potentials, first described by Kuffler *et al.* (1951), are propagated events, or whether intrafusal fibres give rise only to junctional

potentials as in the case of the 'slow' muscle system of the frog (Kuffler & Vaughan Williams, 1953*a*; Burke & Ginsborg, 1956). The present experimental evidence shows that the recorded intrafusal potentials are propagated. In fact, the intrafusal potential as herein recorded showed many of the characteristics of propagation in a volume conductor. More conclusive was the fact that curare blocked these responses in stepwise or all-or-none manner. In addition, physostigmine did not alter the amplitude or time course of these responses. These results eliminate the possibility that the recorded intrafusal potentials were exclusively localized at the intrafusal neuromuscular junction. In curarized spindles intrafusal junctional potentials were not recorded with the method employed.

Boyd (1959) has suggested, on the basis of histological evidence, that 'slow' fibres, similar to those found in amphibians, might occur in the mammalian spindle. In the present study no intrafusal responses have been detected which had the characteristics of 'slow' fibres. By analogy with the frog one would expect that mammalian 'slow' intrafusal junctional potentials would be considerably smaller than propagated responses produced by twitch intrafusal fibres. It is possible that the present recording system was not sensitive enough to detect such small potentials. An unequivocal answer should be obtained by recording intracellularly or close to the spindles with extracellular micro-electrodes.

One might recall here embryological studies on the development of mammalian intrafusal bundles (Cuajunco, 1927, 1940). It seems that intrafusal fibres develop from the same elements which eventually produce the ordinary muscle fibres. In the new-born animal the structure of intra- and extrafusal fibres is similar except that intrafusal elements appear more immature. Later on this difference becomes more striking since extrafusal fibres continue to grow while their intrafusal counterparts do not. It is not surprising, therefore, that intrafusal fibres produce in the adult electric responses similar to those obtained from extrafusal fibres. However, the fact that mammalian intrafusal bundles have an independent nerve supply in contrast to other species has yet to be accounted for.

It is interesting to notice the lack of a clear differential blocking action to curariform agents of the mammalian intrafusal neuromuscular junctions. This contrasts with Katz's (1949) original observation in amphibians. He suggested that the differential sensitivity to curare could be due to a higher safety factor because of a motor fibre making contact with a smaller muscle fibre. In mammals the intrafusal fibres are also smaller than extrafusal ones, but a clear differential sensitivity is not obvious. It seems, therefore, that other factors besides a diameter relationship play a part in differential pharmacological sensitivities to curariform agents. At present it is difficult to envisage such factors since the function and submicroscopic

structure of the post-synaptic membrane of intrafusal junctions are to a large extent unknown.

The action of physostigmine, producing repetitive intrafusal potentials, might be correlated with Hunt's results in the intact animal (1952 and private communication). This author showed that injections of physostigmine increased the effectiveness of fusimotor activation on the afferent discharges. This effect was blocked by curare. It is possible that repetitive intrafusal responses, whether produced by a long-lasting transmitter action or by discharges originating in the nerve terminals, might give rise to stronger and longer-lasting intrafusal contractions, as it occurs in extrafusal fibres.

SUMMARY

The electrical activity of mammalian intrafusal fibres was studied in the excised tenuissimus of the cat and the following was found:

1. Intrafusal potentials recorded from the muscle surface during stimulation of single fusimotor fibres showed a potential sequence which is similar to that of propagated potentials recorded from a volume conductor.

2. Methodical exploration of the muscle in millimetre steps permitted the location of 'active' regions during fusimotor activation. Such regions are probably the site of contracting intrafusal bundles.

3. Fusimotor stimulation produced sometimes complex intrafusal potentials. This complexity is due, in part at least, to potentials originating from different spindles. Complex potentials were more frequently obtained if more than one fusimotor fibre was activated.

4. When a single intrafusal fibre was stimulated with two shocks at varying intervals, the second potential disappeared abruptly when the interval was reduced to 0·7 msec. Also, the onset of the second potential was delayed if the intervals between shocks were less than 3·4 msec.

5. Curare in doses of from 5×10^{-7} (w/v) to 3×10^{-6} (w/v) blocked intrafusal potentials either in stepwise or all-or-none fashion. There is no clear differential sensitivity to curare, Flaxedil or Metubine between extra- and intrafusal junctions.

6. Physostigmine in doses of from 5×10^{-7} (w/v) to 3×10^{-6} (w/v) did not change the amplitude or time course of the intrafusal potentials. Occasionally, intrafusal repetitive responses were produced by this drug.

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THE MOTOR REGULATION OF MAMMALIAN SPINDLE DISCHARGES

By C. EYZAGUIRRE

From the Department of Physiology, University of Utah College of Medicine, Salt Lake City, Utah, U.S.A.

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Stimulation of fusimotor fibres increases the frequency of spindle afferent discharges. Kuffler, Hunt & Quilliam (1951) and Hunt & Kuffler (1951) have shown that this effect is dependent on the frequency of efferent stimulation and on the initial resting tension. Also, the increase in sensory discharge, produced by efferent impulses, is characterized by a graded rise in frequency (facilitation). The time taken to reach peak frequency varies in different units. On cessation of motor stimulation the sensory discharges return to base-line levels either gradually or after an abrupt decrease in frequency ('off effect') which brings the discharge frequency temporarily to values below those existing before stimulation.

Since an increase of sensory frequency elicited by stimulation of fusimotor fibres is almost certainly produced by contraction of the intrafusal bundles, it is important to know how sensory frequency changes are related to intrafusal events. The latter may be schematically described in three main categories: (i) mode of activation of intrafusal muscle fibres, which recent experiments have shown to be through propagated action potentials (Eyzaguirre, 1960); (ii) contractile activity of intrafusal muscle fibres, of which little is known and (iii) the coupling of contractile events and stretch-deformation of the sensory nerve endings.

In this study experiments were designed to analyse the relation between intrafusal potentials and sensory discharges occurring during fusimotor stimulation. The results show that while sensory discharges reach a peak after a facilitation period the intrafusal potential amplitude remains unchanged throughout motor stimulation. Since it has not yet been possible to record intrafusal contraction directly, an indirect method was employed to compare the probable course of intra- and extrafusal contraction. Sensory frequency curves obtained during fusimotor stimulation were constructed and their time course compared with the time course of development of extrafusal muscular tension. These series suggested that, in general, intrafusal fibres are slower than extrafusal elements, in agreement with a previous suggestion of Kuffler & Hunt (1950). Frequency

changes obtained after withdrawing the motor stimulating pulses to the spindles were analysed and related to possible events occurring at the receptor level.

METHODS

In general the methods emplcyed were similar to those presented in the previous paper (Eyzaguirre, 1960). However, in this study muscle tension was recorded; one end of the muscle was attached to a short pin connected to the plate of a RCA 5734 transducer tube while the other end was held rigidly. The natural frequency of the transducer system was about 1 kc/s. In all experiments the temperature of the bath was carefully controlled and it ranged between 37·5 and 38·0° C.

RESULTS

Relationship between intrafusal potential amplitude and sensory discharge facilitation

Kuffler *et al.* (1951) found that the sensory discharge acceleration obtained during fusimotor activation showed marked facilitation. In other experiments they observed that intrafusal potentials showed no increment during repeated excitation, an observation which was confirmed and extended in the previous study (Eyzaguirre, 1960). A more detailed analysis of the sensory discharge facilitation and its possible relation with intrafusal potential amplitude was made by recording both events simultaneously.

Short strains of stimuli were delivered to the fusimotor fibres, the frequency of impulses within the trains ranging from 30 to 200/sec. Figure 1 illustrates one experiment in which four shocks at 100/sec were delivered to a single fusimotor fibre. Discharges of a single sensory fibre were recorded, and frequency curves were constructed and plotted against time. The reciprocal of the interval between two impulses was taken as the instantaneous rate and plotted in time half way between them. The upper part of Fig. 1 illustrates the afferent discharge acceleration obtained from five consecutive records. Fusimotor stimulation increased the sensory discharge frequency from 50 to about 95/sec, reaching a peak in 38 msec after the first shock and 8 msec after the last; after the peak frequency discharges reached base-line levels in about 100 msec. Intrafusal potential amplitudes recorded from fourteen observations are presented in the lower part of Fig. 1. The values obtained for the different intrafusal potentials within the trains are not significantly different.

Figure 2 illustrates another experiment in which one or two fusimotor fibres to the same spindle were stimulated by axon reflex. The contracting intrafusal bundle could be seen near the surface of a very slender muscle. The leading electrode was placed on top of this bundle while the other remained at the crushed end of the preparation. The sensory unit showed no discharge at the resting tension employed. A single nerve shock elicited

a single sensory discharge 8·6 msec after the onset of the intrafusal potential (Fig. 2*A*). Such a sensory response to a single fusimotor shock was uncommon, since in most cases tetanic stimulation was necessary to elicit sensory acceleration. In Fig. 2*B* four pulses were delivered at 30/sec, which elicited three sensory discharges. In Fig. 2*C* seven shocks were applied at 50/sec and four sensory discharges ensued, presenting an approximate shock:discharge ratio of 2:1. In Fig. 2*D* and *E* the stimulus

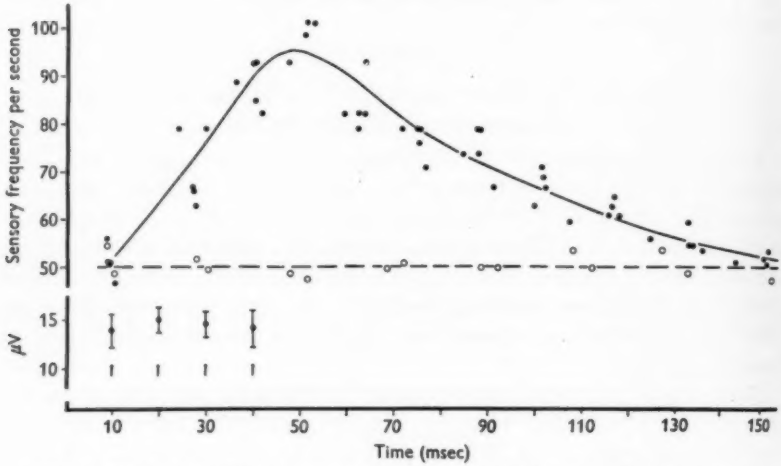

Fig. 1. Effect of fusimotor stimulation on sensory discharges and on intrafusal potentials. Four fusimotor shocks were delivered at 100/sec. Open circles illustrate the basal discharge frequency (broken line). Filled circles (solid line) show afferent frequency changes during intrafusal contraction in five consecutive observations. Arrows indicate the position in time of the shocks. The amplitude of intrafusal potentials after each shock is illustrated above the arrows together with two standard deviations of each observation. The potentials averaged 13·8 μV $\pm$1·65 S.D.; 14·9 μV $\pm$1·33 S.D.; 14·4 μV $\pm$1·26 S.D. and 14·1 μV $\pm$1·99 S.D. in this order. Lower ordinate, amplitude of intrafusal potentials; upper ordinate, sensory frequency per second.

strength was increased; stimulation at 30 and 50/sec produced larger intrafusal potentials and a more efficient sensory acceleration. In fact, at these frequencies and at this stimulus strength, 'driving' of the sensory discharges is clearly seen, with little facilitation. However, it may be noticed in the illustration that the shock–sensory impulse latency of the first discharge in each train is longer than that of subsequent impulses. Also, the shock–discharge latencies of the first impulse in Fig. 2*B* and *C* are longer than those of Fig. 2*D* and *E*. Stimulation of an additional fusimotor fibre (Fig. 2*D* and *E*) produced a larger intrafusal potential and a

more efficient sensory acceleration than stimulation of a single efferent fibre (Fig. 2*B* and *C*). The larger intrafusal potential must have been a complex formed by two smaller components. The overlap and summation of two intrafusal potentials and the increased effect on the sensory discharge indicate that the two fusimotor fibres must have innervated the same spindle.

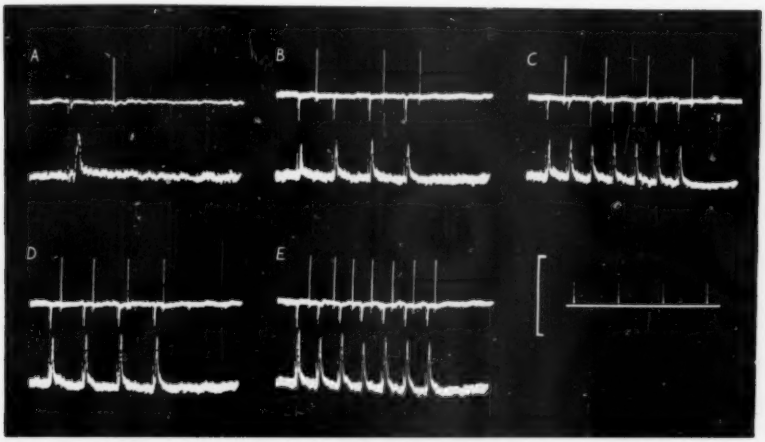

Fig. 2. Relation between intrafusal potentials and increase in sensory frequency during intrafusal contraction; upper beam, sensory discharges recorded from single afferent fibre; lower beam, intrafusal potentials recorded from muscle; fusimotor fibres stimulated by axon reflex. *A*, *B* and *C* illustrate effects obtained by fusimotor stimulation at 1, 30 and 50/sec, respectively. *D* and *E* show effect on sensory discharge and on intrafusal potentials at 30 and 50/sec, respectively, after increasing stimulus strength. Voltage, 100 μV. Time marker, 10 msec for *A* and 20 msec for *B*–*E*.

The experiments illustrated in Figs. 1 and 2 show that facilitation of the sensory discharge during fusimotor activity is dependent on mechanisms within the spindle other than those processes responsible for the intrafusal potential. This is shown by the fact that within a train the intrafusal responses are all of the same amplitude, while sensory discharges undergo marked facilitation. Furthermore, the experiment of Fig. 2 suggests that one of the factors responsible for rate of facilitation of the sensory discharge is the strength of the intrafusal contraction.

Effect of fusimotor frequency of stimulation on sensory discharge facilitation

It has been known for some time that sensory discharge facilitation is dependent on the initial resting tension and on the frequency of fusimotor

impulses (Kuffler *et al.* 1951; Hunt & Kuffler, 1951). The effect of changes in resting tension on sensory discharge facilitation was generally confirmed, but was not systematically studied. However, detailed information was obtained in a number of sensory units which were activated by fusimotor stimulation at different frequencies while the resting tension remained constant. Figure 3 shows the relation between stimulus frequency (abscissa) and increase in sensory discharge above the resting level (ordinate). Sensory discharges were recorded from four single afferent fibres (*A*, *B*, *C*, and *D*). Each point was determined by averaging the peak

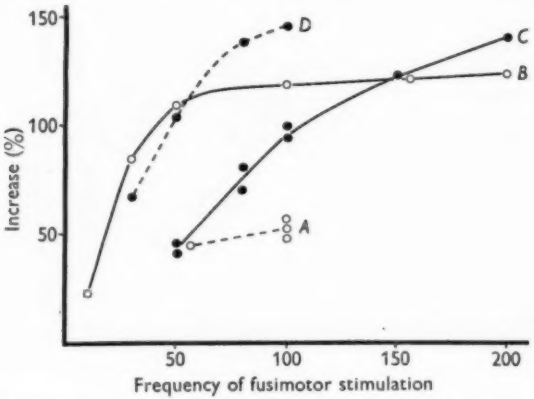

Fig. 3. Effect of fusimotor frequency of stimulation on sensory discharge acceleration; four afferent fibres recorded in different experiments. Unit *A* had a basal discharge rate of 37·6/sec. Unit *B* discharged at 21/sec before stimulation; *C* base-line frequency 31·3/sec; *D* base-line frequency 19·9/sec. Abscissa, frequency of fusimotor stimulation; ordinate, percentage of sensory frequency increase over base-line levels.

frequency (impulses/sec), stimulation being continued until after the peak frequency was reached. Between runs sufficient time was allowed for sensory discharges to become stabilized at base-line levels. It may be seen that the sensory discharge acceleration increased with the frequency of efferent stimulation. However, at certain frequencies of stimulation, some sensory fibres reached a 'saturation' point, as exemplified in the experiment illustrated in curve *B*. This unit showed an increase of 110% over base-line levels when the fusimotor fibre was stimulated at 50/sec and an increase of 123% when the frequency of fusimotor stimulation was raised to 200/sec. Units *C* and *D* would, presumably, have reached a similar plateau at higher frequencies of stimulation.

The experiments just described suggest that at a given resting tension the peak sensory frequency developed during fusimotor activity is related to

the frequency of the efferent impulses (see also Fig. 6). 'Driving' of the sensory discharges, as described in other muscles (Kuffler *et al.* 1951), was infrequently observed in this preparation. An example of this is provided in Fig. 2 *D* and *E* and in curve *C* of Fig. 3. In the latter experiment driving was established at efferent frequencies between 50 and 100/sec at the resting tension employed. At higher efferent frequencies this effect was not evident.

A comparison of the facilitation period of sensory discharges during fusimotor activity and the time course of development of extrafusal tension

Kuffler *et al.* (1951) showed that sensory discharges reached a peak of facilitation, during motor spindle activation, some time after the onset of stimulation. This lapse of time has been called the facilitation period.

The term facilitation period will be employed here as the lapse of time necessary for the sensory discharges to increase from base-line levels to a value of 70% of the sensory frequency peak obtained during fusimotor activity. This arbitrary value has been chosen because very often the constructed frequency curves were frankly asymptotic and the time from the onset of stimulation to the frequency peak was difficult to determine.

The facilitation period of sensory discharges during fusimotor activation varied in different units. Some fibres reached 70% of the firing peak shortly after onset of stimulation, whereas others did so only after a relatively long period of time. The time required to reach this value was dependent on the frequency of the efferent train. Some examples are illustrated in Fig. 4, in which the same units described in Fig. 3 are plotted. The abscissa represents the frequency of motor stimulation to the spindles and the ordinate the time required to reach 70% of the peak sensory frequency. It may be seen that in all units the higher the frequency of stimulation the shorter was the facilitation period. At low frequencies of stimulation the facilitation period was sometimes quite long; such a case is illustrated in curve *B* of Fig. 4. Stimulation of the fusimotor fibre at 10/sec accelerated the sensory discharge with a facilitation period of 1 sec. At 30/sec this time was shortened to 277 msec and at 50/sec the period of facilitation lasted for 202·6 msec. Units *C* and *D* had shorter facilitation periods than *B*. Thus, fusimotor stimulation at 30/sec produced a facilitation period of 51·2 msec in unit *D*. Stimulation at 50/sec determined a period of facilitation of 132·5 msec in unit *C* and of 16·3 msec in unit *D*. Fusimotor stimulation at 100/sec elicited a facilitation period which varied from 110 to 172·3 msec in unit *A*; this period was of the order of 145·8 msec in unit *B* and lasted for 93·1 and 16·0 msec, respectively, in units *C* and *D*.

Figure 4 shows that the several sensory units obtained from different preparations showed facilitation periods which were dependent on the frequency of fusimotor stimulation in spite of differences in individual

units. The effect of resting tension on the facilitation period of different sensory units was not systematically studied. One would suspect, however, that the initial tension, which influences the sensory discharge burst during motor spindle stimulation, must also influence the facilitation period. Such effect is clear in other species (Eyzaguirre, 1958).

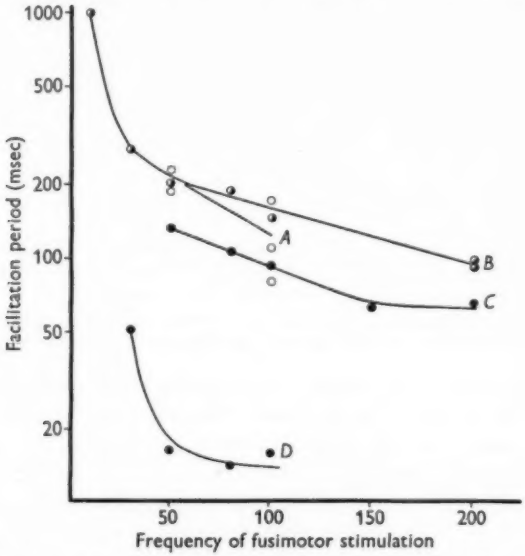

Fig. 4. Facilitation period of sensory discharges during fusimotor stimulation at different frequencies. Units also analysed in Fig. 3. Semi-log. scale.

Since sensory discharge changes during spindle activation are in all probability the result of intrafusal contractile events, it would be of interest to compare intra- and extrafusal contractile changes. This study was handicapped by the lack of an adequate technique to measure intrafusal contractions. Consequently, an indirect method was employed, namely, the use of the facilitation period as an index of the speed of intrafusal contraction. This criterion, with some qualifications (see p. 193), is probably adequate, since the spindle output reflects events occurring after application of an external load (Matthews, 1931; Katz, 1950) and presumably intrafusal contractile changes. Muscle tension was recorded during maximal motor nerve stimulation to the tenuissimus (see Methods). The muscle resting tension was adjusted so as to produce maximal tension during single and repetitive nerve shocks. Frequency of stimulation ranged between 10 and 200/sec. Between runs a resting period of several minutes was allowed.

Figure 5 illustrates the effect of different frequencies of stimulation (abscissa) on the time required for the muscle tension to reach 70 % of its peak (ordinate) from three different experiments. It may be seen that in all cases this time was shorter when the frequency of motor stimulation was higher. If one compares the values obtained in Fig. 4 with those of Fig. 5 it appears, in general, that intrafusal contraction (judged by sensory frequency curves) is slower than extrafusal tension changes. This is particularly striking in unit *B* of Fig. 4, where fusimotor stimulation at 10/sec was associated with a facilitation period of 1 sec. This contrasts with extrafusal tension development, since in all muscles studied, at that

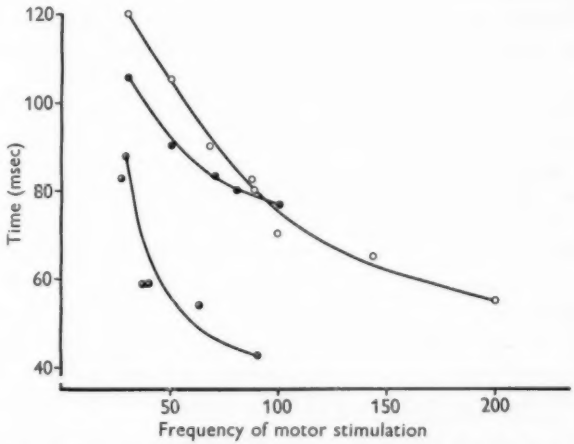

Fig. 5. Effect of frequency of motor stimulation on the rate of extrafusal tension development; curves constructed from three different experiments. Resting tension in each case was adjusted to produce maximal contraction during single and repetitive nerve shocks. Abscissa, frequency of motor stimulation. Ordinate, time required to reach 70 % of tension peak.

frequency of stimulation, the contractions were not fused and individual twitch responses were recorded. Each twitch response had a time course of about 15 msec to 70 % of its peak (see Discussion). The frequency changes of some sensory units, however, indicated that intrafusal contractile events could occasionally be faster than those of extrafusal fibres (see unit *D* in Fig. 4).

The above bases for comparison between extra- and intrafusal elements are probably insufficient. They were used only to gain an approximate estimate of contractile events in both systems. The actual time courses of intra- and extrafusal contractile changes are likely to be different from values obtained from the sensory frequency curves and from muscle

tension changes. The mechanical coupling during intrafusal contractions is likely to be quite effective in producing stretch-deformation of the endings, as sensory discharge acceleration occurs during rapid application of an external load (Katz, 1950). Furthermore, it is not known to what extent the dynamic effects of stretch may influence the sensory discharge frequency during the rise in intrafusal tension. The time course of the recorded changes in extrafusal tension will also be influenced by series visco-elastic components. Hill (1949) has shown that mechanical changes in muscle start before tension changes are detected by conventional transducers. This damping is probably quite appreciable in the tenuissimus, since all muscle elements are placed serially along the longitudinal axis. A more accurate estimate of intrafusal contractile changes should be obtained by recording tension directly from a contracting spindle.

Sensory frequency changes after cessation of fusimotor activation

Kuffler *et al.* (1951) have reported that on cessation of stimulation of fusimotor fibres the accelerated spindle discharge may follow either one of two different patterns: (i) they may decrease gradually to the base-line level or (ii) they may abruptly fall to levels below the original base line and then return gradually to the previously existing resting frequency ('off effect'). These authors also described post-excitatory facilitation in the form of sensory discharge frequencies above the base-line level after withdrawal of the stimulating pulses. At the same time they found phenomena resulting from the combination of these different situations. These observations were confirmed and extended in the present study.

The 'off effect'

A complete cessation of sensory discharges occurring immediately after withdrawing fusimotor trains was observed only in relaxed preparations in which the basal discharge rate was low. More frequently a fall in frequency to a variable extent below the base-line frequency was obtained. Figure 6 illustrates an experiment in which discharges were recorded from a single afferent fibre. A single fusimotor fibre was stimulated at frequencies of 50 (*A*), 80 (*B*), 100 (*C*), 150 (*D*) and 200/sec (*E*), the duration of the stimulating trains being 4 sec in each case and the initial tension remaining constant. Between runs a resting period of about 2 min was allowed for the discharges to reach base-line levels. The basal frequency was about 30/sec. This illustration clearly shows that the frequency of sensory discharges reached higher levels with higher frequencies of fusimotor stimulation. Thus the frequency of sensory discharges increased only to 39/sec when the fusimotor fibre was stimulated at 50/sec (*A*); the highest frequency peak was obtained when the spindle was activated at 200/sec (*E*).

In this last case the sensory discharges reached 71/sec. The upper broken line links those points indicating the frequency at which the receptor was firing at the time of stimulation withdrawal. The lower broken line connects the points in each series representing minimal frequencies after stimulation.

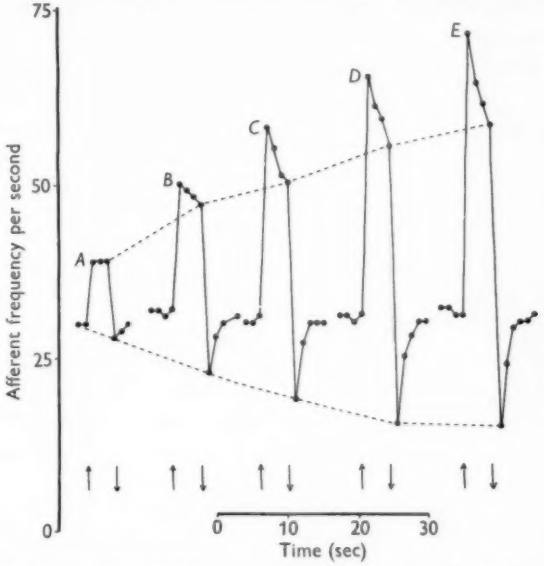

Fig. 6. 'Off effect' produced at the end of fusimotor stimulation. Between upward and downward arrows a single fusimotor fibre was stimulated for 4 sec; between runs a resting period of 2 min was allowed. *A* stimulation at 50/sec; *B* at 80/sec; *C* at 100/sec; *D* at 150/sec; *E* at 200/sec. Upper broken line connects points indicating afferent frequency at the moment of stimulation withdrawal; lower broken line connects points of minimal sensory frequency.

The experiment illustrated in Fig. 6 shows that the magnitude of the 'off effect' is closely related to the frequency of the sensory discharges at the moment of stimulation withdrawal. The fall in frequency was not appreciably greater in relaxed than in stretched preparations. In the same experiment the initial tension was reduced, resulting in a fall of the basal discharge frequency from 30 to 17/sec (not illustrated). Efferent stimulation similar to that applied in Fig. 6*B* (80/sec for 4 sec) now caused the sensory discharge frequency to reach 33/sec by the end of the stimulating train, and to fall to a minimum of 11/sec after cessation of stimulation. This amounts to a fall in frequency of 22 impulses/sec, which is not significantly different from that observed in Fig. 6*B* where a fall of 24 impulses/sec was recorded. With the muscle at the same reduced initial tension, complete cessation of sensory discharge followed fusimotor stimulation at 100/sec.

In general it was easier to obtain complete receptor silence after fusimotor stimulation when the preparation was under low initial tension.

The observed 'off effect' may be explained by assuming that hyperpolarization of the sensory nerve endings occurs during intrafusal relaxation. This effect would be similar to that obtained during sudden release of a stretched receptor organ (Katz, 1950; Eyzaguirre & Kuffler, 1955). If this is the case, it is reasonable to assume that hyperpolarization of the

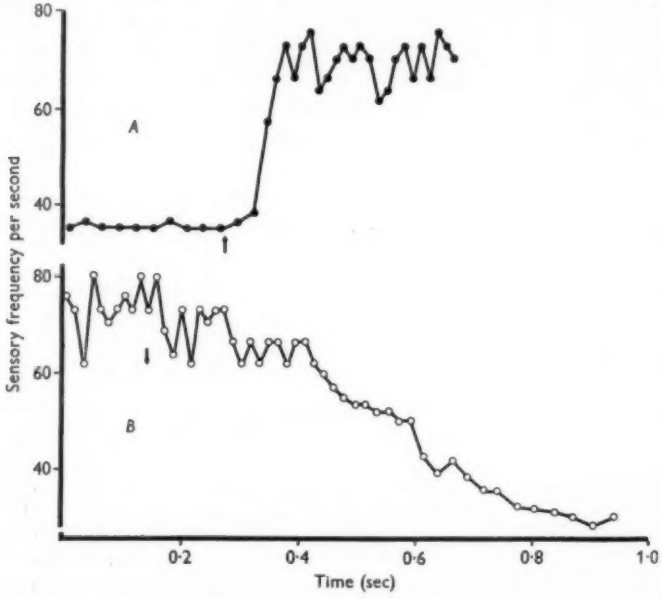

Fig. 7. Effect of fusimotor stimulation on sensory discharge frequency. *A*, filled circles show effect on sensory frequency produced by fusimotor activity at 50/sec; upward arrow indicates onset of stimulation which was maintained for 1·3 sec. *B*, open circles illustrate decrease of sensory discharge frequency after withdrawing stimulation (downward arrow).

sensory endings during abrupt relaxation of a contracting intrafusal bundle may cause the generator potential to fall below the firing level, as in the case of a receptor under little initial tension. If, on the other hand, intrafusal contraction occurs at a higher resting tension, hyperpolarization of the afferent terminals may not be sufficient to result in a fall of the generator potential below the firing level. Consequently only a slowing of the sensory discharges will be observed. Similar 'off effects' have been recorded after contraction of the twitch intrafusal system of amphibians (Eyzaguirre, 1957, 1958).

In several experiments sensory frequency increased rather abruptly during fusimotor activation but fell gradually after withdrawing the stimulating pulses. Such an observation is illustrated in Fig. 7. The base-line discharge frequency of the receptor was about 35/sec. In *A*, a single fusimotor fibre was stimulated at 50/sec (upward arrow) and stimulation was maintained for 1·3 sec; sensory discharges reached a peak in 76 msec (filled circles). *B* illustrates the sensory discharge frequency after stimulation withdrawal (downward arrow); sensory discharge frequency reached base-line levels in 0·6 sec but continued to decrease for another 160 msec; at this point sensory frequency reached its maximal undershoot when the receptor discharged at 27/sec (open circles). The receptor recovered but the discharge reached base-line levels only after several seconds. This prolonged and late depression following fusimotor activity was frequently observed when sensory discharges tapered off gently after intrafusal contraction. It is difficult to explain this late effect, particularly when the sensory discharge increase at the onset of contraction showed a steep rising curve. In this case the presence of visco-elastic elements interposed between the contractile component and the receptor cannot be the sole cause, since one would expect also a slowing down of the initial part of the frequency curve.

DISCUSSION

One of the principal aims of this study was an analysis of sensory discharge facilitation during fusimotor activity and its possible relation to intrafusal events. Facilitation cannot be attributed to changes in the mechanisms that elicit the intrafusal potentials. These potentials remain of constant amplitude during fusimotor activity while sensory discharges undergo marked facilitation. Several other factors could be responsible for the presence of facilitation, such as the strength and time course of intrafusal contraction and the mechanical coupling of the sensory nerve endings with the contractile elements.

The strength and the time course of intrafusal contraction must have some influence in determining facilitation. The effect of strength of intrafusal contraction is suggested in the experiment illustrated in Fig. 2. When more than one fusimotor fibre was stimulated the intrafusal potentials became larger and the latencies of individual sensory discharges during the fusimotor train shorter. Also, the increase in sensory discharge frequency was greater at higher stimulating frequencies, which would indicate that at higher efferent frequencies of stimulation stronger intrafusal contractions occurred. In addition, higher stimulating frequencies shortened the facilitation period, an effect which is similar to changes in the rate of development of tension of extrafusal fibres during nerve stimulation. Consequently, one may assume that a shorter facilitation period means faster development

of tension in intrafusal fibres. It seems, however, that the facilitation period cannot be explained solely on the basis of development of intrafusal tension during fusimotor stimulation. This point is particularly well exemplified in an instance in which one fusimotor fibre was stimulated at 10/sec and the resulting facilitation period lasted for about 1 sec (Fig. 4*B*). In this case it is difficult to envisage facilitation as produced only by intrafusal contractile events, especially if the intrafusal fibres produce propagated action potentials. Consequently other factors must play a part in the production of facilitation, such as the mechanical coupling of the sensory endings and intrafusal contractile components. However, at present there is so little direct information about stretch-deformation of the endings during intrafusal contraction that it is difficult to discuss the problem. In amphibians the sensory discharges elicited by activation of the twitch intrafusal fibres appear early after the onset of the intrafusal action potential, indicating that mechanical coupling does not introduce a significant delay in the onset of stretch-deformation of the endings (Eyzaguirre, 1957). But it is clear that this may not be the case in mammalian spindles.

A comparison of the time course of sensory frequency curves and the rate of extrafusal tension development during repeated nerve stimulation seems to indicate that intrafusal fibres are, with some exceptions, generally slower than extrafusal ones. This observation agrees with a previous suggestion of Kuffler & Hunt (1950), based also on the discharge pattern obtained during fusimotor activity.

Variations in the facilitation period following fusimotor stimulation in different sensory units could be due to several factors: (i) recording of discharges from fibres belonging to either Groups Ia or II, since it is known that they are connected to annulospiral or flower-spray endings respectively (Barker, 1948; Hunt, 1954); (ii) stimulation of different types of intrafusal muscle fibres and (iii) differences in mechanical coupling of nerve endings and contractile elements. The first possibility is unlikely, since Hunt (1954) has demonstrated that no significant variations in the response of these fibres to fusimotor stimulation occurred in a large sample of fibres. As to the second, Boyd (1959) has suggested, on the basis of histological evidence, that more than one type of intrafusal fibre occurs in compound spindles. However, intrafusal potentials recorded in these experiments behaved as propagated responses in all the cases studied, whether sensory frequency response to fusimotor stimulation showed a fast or a slow time course. It seems, therefore, that the variations observed in the different frequency curves cannot be attributed to the presence of 'slow' and 'fast' intrafusal fibres as is the case in amphibians (Eyzaguirre, 1957, 1958). One cannot exclude, however, the possibility that the speed of contraction of

different intrafusal fibres may vary in different spindles in a quantitative manner. The third possibility, differences in coupling, is likely to have some influence on facilitation, since some nerve endings could be engaged readily during intrafusal contraction, whereas in others, owing to a greater visco-elastic component linking the contractile process to the nerve terminal, a longer time may be required to produce stretch-deformation of the ending and hence a rise in sensory frequency.

SUMMARY

1. Intrafusal potentials were recorded simultaneously with sensory discharges from single afferent fibres during fusimotor activation at different frequencies. The intrafusal potential amplitude remained constant during stimulation while the sensory discharges showed appreciable facilitation.

2. Stimulation of more than one fusimotor fibre produced larger intrafusal potentials than stimulation of a single fusimotor fibre. This effect was accompanied by more effective sensory acceleration.

3. The maximal sensory frequency obtained during fusimotor activity was dependent on the frequency of the efferent stimulation.

4. Fusimotor activation produced an increase of sensory discharges after a period of facilitation. The period of facilitation was dependent on the frequency of the fusimotor impulses.

5. The time required for extrafusal muscle tension to reach 70% of its peak during tetanic nerve stimulation was generally shorter than the time required by sensory units to reach 70% of the frequency peak during fusimotor stimulation.

6. On cessation of fusimotor stimulation the increased sensory discharges returned to base-line levels either gradually or abruptly ('off effect'). In the latter instance the magnitude of the 'off effect' was related to the frequency of the sensory discharges at the moment of stimulation withdrawal.

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BARORECEPTOR REFLEXES IN NEW-BORN RABBITS

BY S. E. DOWNING
From the Nuffield Institute for Medical Research, University of Oxford

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This paper describes an investigation of the baroreceptor reflexes from the carotid sinus and aortic arch in the new-born rabbit. Bauer (1939) concluded from measurement of the heart rate in rabbits that the depressor reflex was not activated during asphyxia until the 30th day from birth, and the carotid sinus reflex until the 40th day. He also stated that 'faradic stimulation of the depressor nerves and carotid sinus regions in rabbits produces a reflex inhibition of the heart as early as the 11th and 14th days, respectively'. Both Bauer (1939) and Barcroft (1946) inferred from these and other observations in Bauer's paper that the threshold pressures for the baroreceptor reflexes were high, 65 mm Hg for the depressor and 80 mm Hg for the carotid sinus reflex; the normal blood pressure of a new-born rabbit did not reach these levels until several weeks from birth. The implication is that in the new-born rabbit the cardiovascular system is not under the control of the baroreceptors unless the blood pressure rises above the threshold levels quoted above. The object of the experiments now to be described was to determine by more direct methods whether the baroreceptor reflexes are functioning in the rabbit during the first few days of life at the low arterial pressure which is then normally present.

METHODS

Observations were made on seventeen adult, nine young (3-weeks-old or more, 0·2–1·0 kg body weight) and seventy-five new-born (0–15 days) rabbits, anaesthetized with pentobarbitone and/or urethane. Of the new-born rabbits fifty-five were given pentobarbitone (10–15 mg/kg intraperitoneally, supplemented with additional pentobarbitone as necessary), and the remainder received urethane (1·2 g/kg intraperitoneally). Older rabbits were given pentobarbitone (20–25 mg/kg intravenously) followed by urethane (1·2 g/kg subcutaneously). The trachea was cannulated. In most experiments rectal temperature was recorded by a thermocouple, and was maintained at $38 \pm 1°$ C by application of external heat as necessary. This was found to be particularly desirable in very young rabbits.

Pressure changes applied to the carotid sinus. In new-born rabbits the left carotid sinus was isolated from the rest of the circulation. The cardiac end of the right or left common carotid artery was cannulated to record systemic blood pressure. The cephalic end of the left common carotid artery was connected by a polyethylene tube to a pressure bottle filled with saline and to a manometer. The left external carotid was tied, together with all branches of the common carotid above the polyethylene tube and the internal carotid distal to the sinus. Static and pulsatile pressures were applied to the carotid sinus. Pulsations were

generated by an armature, activated by an electromagnet, which compressed the soft plastic tube connecting the pressure bottle with the carotid sinus, at a frequency which was adjusted to about 180 cycles a minute.

The Valsalva manoeuvre. The endotracheal pressure was raised for short periods of time in the following way. The tracheal cannula was attached to a T-tube through which a constant stream of air was passed from a roller-pump freely into the atmosphere. At any moment this stream of air could be diverted through a glass tube immersed to a depth of 5, 10 or 15 cm in water.

Records of blood pressure and nerve action potentials. Single and multifibre preparations of the aortic depressor, carotid sinus and vagus nerves were made by conventional methods. The action potentials were amplified and displayed on a cathode-ray oscilloscope together with the arterial blood pressure, recorded from a polyethylene catheter in the cardiac end of the right carotid artery by a condenser manometer. When critically damped, the catheter and manometer assembly used for observations on new-born rabbits had a frequency response of 120 c/s. No great difficulty was found in cannulating the carotid arteries of new-born rabbits under a binocular microscope ($\times 16$), with a cannula drawn out from a piece of polyethylene to have an external diameter at the tip of 0·78 mm and an internal diameter of 0·63 mm. Various nerves were stimulated with a constant-current rectangular pulse generator.

RESULTS

Blood pressure and heart rate of rabbits 0–15 days old

The mean blood pressure of eight rabbits which were about 12 hr old was 36 mm Hg (Fig. 1). During the next 2 weeks the blood pressure rose by about 3 mm Hg a day. The heart rate also rose during the first few days after birth, from about 250 beats a minute to 330 or more by the fifth day from birth. There was no significant difference between rabbits anaesthetized with urethane or with pentobarbitone.

Electrical stimulation of the depressor and carotid sinus nerves and of the peripheral end of the vagus

Maximal stimulation of the central end of the left depressor nerve of rabbits less than 6 days old, anaesthetized with pentobarbitone, caused a mean fall in blood pressure of $17 \pm 2{\cdot}5$ (S.E.)% and in heart rate of $7 \pm 1{\cdot}7$%. An example is shown in Fig. 2. In rabbits of the same age anaesthetized with urethane the mean fall in blood pressure ($0{\cdot}6 \pm 0{\cdot}6$%) and heart rate (2 ± 1%) were negligible. In rabbits 6–15 days old under urethane anaesthesia stimulation of the left depressor nerve caused a significant fall in blood pressure (17 ± 4%) and in heart rate ($8{\cdot}5 \pm 3$%). Younger rabbits therefore seem very susceptible to urethane anaesthesia.

Stimulation of the central end of the carotid sinus nerve of rabbits less than 6 days old caused two types of response similar to those described by Douglas & Schaumann (1956) in adult cats. With stimuli of 3–5 mA (at a frequency of 13/sec and duration 5·4 msec) there was an average rise of blood pressure of nearly 30%, a large increase in respiratory rate and some slowing of the heart. When the current was increased to 7·5 mA there

was sometimes a small fall in blood pressure and heart rate (7–10%), but respiratory rate still doubled. It was thus sometimes possible to convert a pressor to a depressor response by increasing the strength of the stimulus. In contrast to the effects of depressor nerve stimulation, the response was not affected by the use of urethane in place of pentobarbitone.

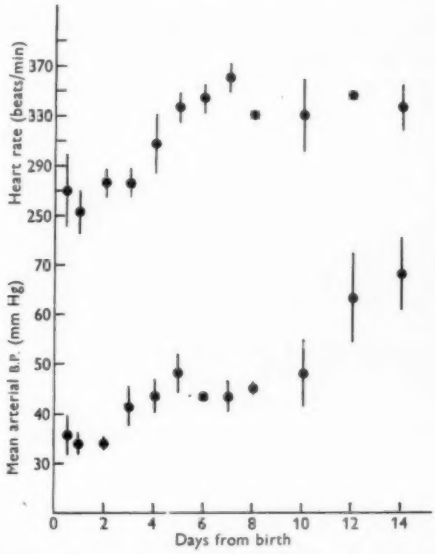

Fig. 1. Observations of heart rate (above) and mean arterial blood pressure (below) from sixty-two rabbits to show the changes during the first 2 weeks of life. The vertical lines represent the standard error of the mean.

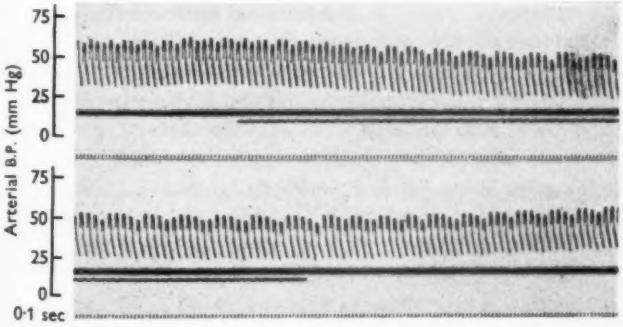

Fig. 2. Rabbit 55 g, 12 hr old, pentobarbitone anaesthesia. Stimulation of the central end of the left depressor nerve during signal mark (continuous record).

Stimulation of the peripheral end of one vagus nerve in fifteen new-born rabbits caused a mean fall in blood pressure of 45 % and in heart rate of 65 %. There was no difference whether pentobarbitone or urethane were used for anaesthesia.

Section of the depressor and carotid sinus nerves

In the following experiments blood pressure was recorded from the cardiac end of the right common carotid artery which was tied; the right carotid sinus nerve was intact. In rabbits less than 6 days old section of the right depressor nerve alone caused only a trivial rise in mean blood pressure. When the left depressor nerve was then cut there was a rapid

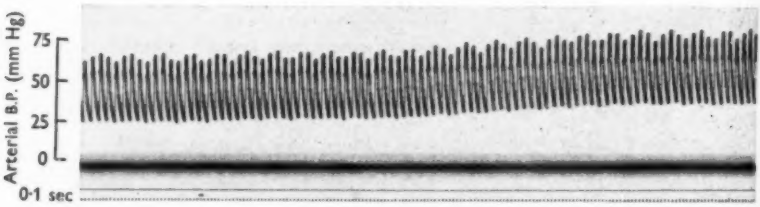

Fig. 3. Rabbit 78 g, 3 days old, pentobarbitone anaesthesia. The left depressor nerve was cut at the signal mark (the right depressor had been cut previously).

rise of $13 \pm 2{\cdot}4$ % under pentobarbitone anaesthesia (Fig. 3), and of $7 \pm 4{\cdot}1$ % under urethane. But as in the adult this rise of pressure was not maintained; the pressure returned to its previous level during the next 5–15 min. When the left carotid sinus nerve was cut, after section of both depressor nerves and when the pressure had returned to its initial level, there was a rise of 12 ± 7 % under pentobarbitone anaesthesia. Once again, after an interval of some minutes, the pressure returned to its initial level. There was no change of respiratory rate. There was little immediate change in the heart rate during any one of these manœuvres, though it usually increased by 15–20 % during the course of the whole experiment.

In similar experiments on three adult rabbits, section of both depressor nerves and the left carotid sinus nerve caused a rise of blood pressure, which returned to its original level over a period of 15–20 min. There was only a small change in heart rate.

Occlusion of the carotid arteries

The right carotid artery was tied and the blood pressure was recorded from its cardiac end in rabbits less than 6 days old. Temporary occlusion of the left carotid caused a mean increase in arterial pressure of $19 \pm 4{\cdot}5$ %

under pentobarbitone, but of only $2{\cdot}7 \pm 2{\cdot}6\%$ under urethane. There was no significant change in heart rate. After cutting both depressor nerves in rabbits under pentobarbitone there was still a rise of $17 \pm 5{\cdot}9\%$ when the left carotid artery was occluded. But when the left carotid sinus nerve was also cut, the rise of pressure was reduced to $5 \pm 3{\cdot}5\%$. This demonstrates that the rise of pressure on occlusion of the left carotid artery was mainly due to a reflex arising in the left carotid sinus, rather than to a rise of systemic resistance due to the mechanical interruption of flow up the left carotid.

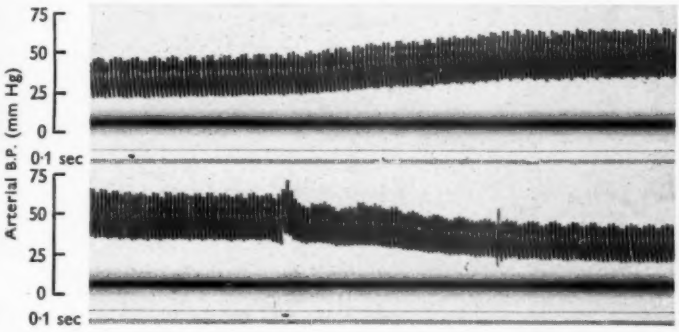

Fig. 4. Rabbit 78 g, 4 days old, pentobarbitone anaesthesia. The left common carotid artery was occluded at the first signal mark (the external, internal and muscular branches had been tied previously), and was released at the second signal mark (continuous record).

When the main carotid artery is occluded, the pressure in the carotid sinus does not fall to zero, because of anastomotic connexions with the rest of the circulation. In six rabbits less than 6 days old, under pentobarbitone anaesthesia, the left external and internal carotid arteries were tied, together with an occasional muscular branch. When the left common carotid artery was then occluded, the arterial blood pressure (recorded from the central end of the right carotid artery) rose by $23{\cdot}5 \pm 8{\cdot}5\%$ (Fig. 4). This evidence also supports the view that the rise of systemic pressure is due to a carotid sinus reflex rather than to an increase in systemic arterial resistance, However, under these conditions the possibility of the response being due in part to activity of the carotid body chemoreceptors (because of anoxia from lack of blood) cannot be excluded.

Raising carotid sinus pressure

In the following experiments blood pressure was recorded from the cardiac end of the left or right common carotid artery. The cephalic end of

the left common carotid artery was connected to a manometer and pressure bottle filled with saline, so that the pressure in the left carotid sinus, isolated from the rest of the circulation (see Methods) could be varied. Nine experiments were carried out on rabbits from 12 hr to 9 days old. In every experiment raising the pressure in the left carotid sinus caused a significant fall in blood pressure; there was little change in heart rate (Fig. 5). When a static pressure of 30 or 40 mm Hg was applied to the

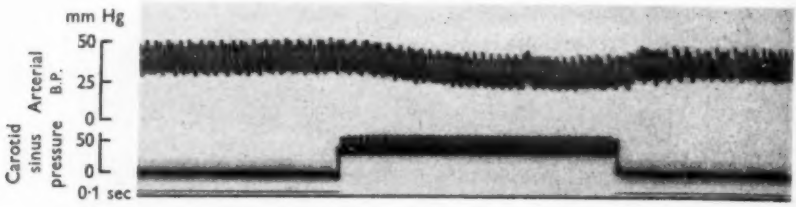

Fig. 5. Rabbit 62 g, 12 hr old, pentobarbitone anaesthesia. Above, arterial blood pressure; below, pressure in the vascularly isolated left carotid sinus.

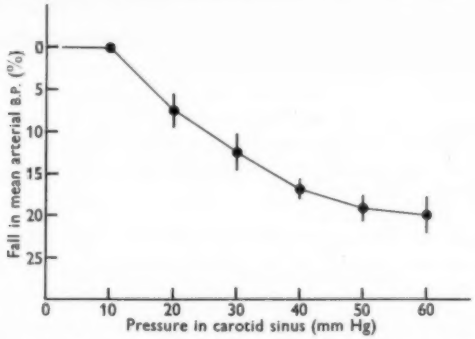

Fig. 6. Observations from nine new-born rabbits to show the fall in mean systemic blood pressure (%) at different pressures applied to the isolated left carotid sinus. The vertical lines represent the standard error of the mean.

carotid sinus, the fall in systemic arterial pressure was significantly less than when a pulsatile pressure of the same mean value was applied; at higher pressures no difference was observed. The rise in pressure required was not large. Thus in one 6-day-old rabbit, raising the pressure in the left carotid sinus from 0 to 41/19 mm Hg (pulsatile) caused the systemic arterial blood pressure to drop from 63/35 to 52/25 (fall in mean B.P. 23 %). When a static pressure of 30 mm Hg was applied to the sinus, arterial pressure only fell from 61/33 to 59/33. A positive correlation between the level of induced carotid sinus pressure and fall in systemic

arterial pressure was observed (Fig. 6). While these changes are not large, it should be remembered that only *one* carotid sinus was being stimulated in these experiments. Denervation of the sinus in five animals completely abolished the response.

The Valsalva test

The characteristic cardiovascular response to a rapid elevation of endotracheal pressure (and hence intrapulmonary pressure) in the adult rabbit consists of a sharp fall in systemic arterial blood pressure followed by a gradual rise. When the endotracheal pressure is returned to atmospheric the blood pressure rapidly overshoots its initial level and thence slowly returns. In the adult rabbit the gradual rise in pressure during the Valsalva manoeuvre, and the overshoot, are dependent on the integrity of the baroreceptors (Joan C. Mott, unpublished). An example is shown in Fig. 7.

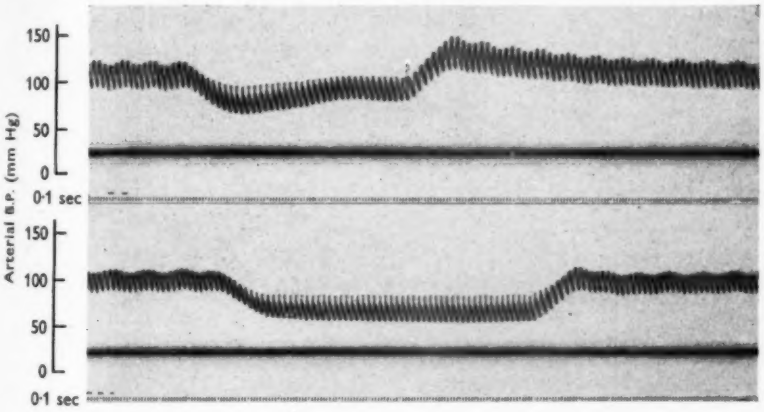

Fig. 7. Rabbit 625 g, 32 days old, urethane anaesthesia. The upper tracing shows the characteristic blood pressure response to rapid elevation of the endotracheal pressure to 15 cm H_2O (Valsalva manoeuvre). The lower tracing shows the response after cutting both depressor nerves.

In only eight of twenty-one rabbits less than 15 days of age was the same sequence of arterial pressure changes clearly seen when the endotracheal pressure was raised and lowered. In the others the responses were equivocal. There was no obvious relation between age and the unequivocal demonstration of these responses. In three out of six new-born rabbits which showed a positive response, cutting the depressor nerves greatly reduced or abolished both the rise in pressure during the Valsalva manoeuvre and the subsequent overshoot.

Baroreceptor nerve activity

Multiple and single fibre preparations were made from the depressor and carotid sinus nerves in adult, young and new-born rabbits. In every animal multiple nerve activity synchronous with the pulse wave was recorded. It was more difficult to obtain good single-fibre preparations in new-born rabbits, as the nerves are so much more fragile. Figure 8*a* shows the record from a whole depressor nerve in a one-hour-old rabbit, and it is evident that there are numerous discharges coincident with the systolic pressure wave; the mean pressure was 33 mm Hg. Figure 8*b* shows a typical multifibre preparation from the depressor nerve of a

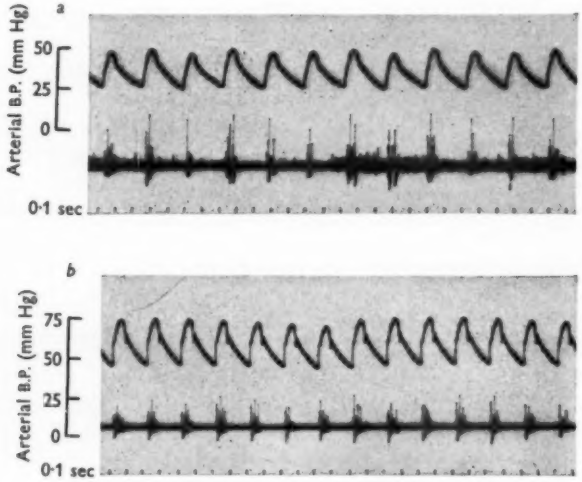

Fig. 8. The upper record (*a*) shows the arterial blood pressure, and action potentials from the entire left depressor nerve of a 38 g, one-hour-old rabbit. The lower record (*b*) shows a typical multifibre preparation from the depressor nerve of a 70 g, 4-day-old rabbit.

4-day-old rabbit; the mean pressure was 50 mm Hg. Numerous preparations of this type were obtained in rabbits 1 hr old and upwards. Figure 9 shows single fibre baroreceptor discharges from the left depressor nerve of a 2-day-old rabbit, at different arterial pressures. In adult rabbits the arterial pressure was altered by haemorrhage or by transfusion of heparinized blood; in the new-born rabbit it was raised by compression of the abdomen. In addition, new-born rabbits usually showed a variation in arterial pressure with breathing. In all instances the frequency of discharge increased with an increase in arterial pressure, and vice versa.

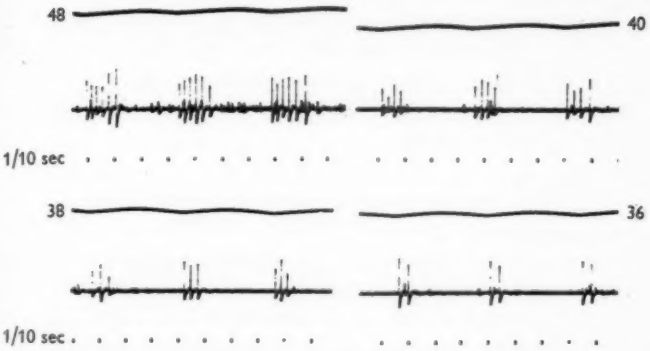

Fig. 9. Records of single baroreceptor discharges from the left depressor nerve of a 68 g, 2-day-old rabbit at four different blood pressures. In each record the arterial pulse is heavily damped and the mean pressure is indicated in mm Hg (upper trace); the middle trace shows the baroreceptor discharges, and the lower trace time.

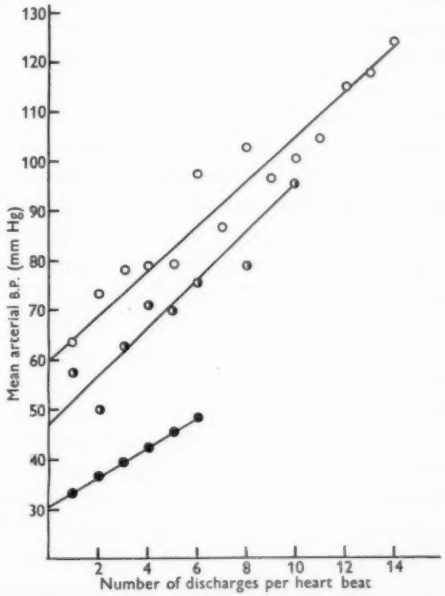

Fig. 10. Observations of single nerve fibre activity from the depressor nerve. The discharge frequency per heart beat of fourteen fibres from adult rabbits (○), eleven fibres from young rabbits, 200–1000 g (◑), and four fibres from new-born rabbits, < 2 weeks old (●), plotted against the mean arterial blood pressure.

Figure 10 shows the number of discharges in each heart beat plotted against *mean* blood pressure for a number of baroreceptor nerve fibre preparations in adult, young and new-born rabbits. It is evident that baroreceptor afferent nerve fibres can discharge at the low arterial pressure of a new-born rabbit. The observations also suggest that the threshold in older rabbits may be higher.

DISCUSSION

There is no doubt that the central and efferent mechanisms for actuation of the baroreceptor reflexes are present in the rabbit at birth. Electrical stimulation of the central ends of the depressor and carotid sinus nerves caused a fall of blood pressure and heart rate when pentobarbitone was used as the anaesthetic. The afferent side of the reflexes was also studied in an attempt to obtain a measure of the threshold pressures at which the reflexes were brought into operation. It proved more difficult to obtain baroreceptor single-fibre preparations from the depressor and carotid sinus nerve in new-born rabbits than in adults. However, the observations on multi-fibre preparations clearly indicated that many afferent nerve fibres were discharging synchronously with each heart beat as early as 1 hr. after birth.

When both depressor nerves were cut in the new-born rabbit there was a rise of blood pressure. When the left carotid sinus nerve was then cut there was also a rise of blood pressure. These observations suggest that there was considerable afferent activity in the nerves before section. Mechanical stimulation of the central end of the depressor nerve at the time of section would have caused a fall of blood pressure. Mechanical stimulation of the carotid sinus nerve might have caused a rise of blood pressure, as does electrical stimulation with a low current, but it is improbable that the rise would have lasted as long as was observed, and the increase in respiratory rate (usually seen during electrical stimulation of this nerve) was not present. There must, therefore, be tonic activity of the baroreceptor reflexes in the rabbit during the first few days after birth.

It will be recalled that Schmidt (1932), Gernandt (1946) and Neil, Redwood & Schweitzer (1949) all found that in rabbits the depressor nerve does not contain chemoreceptor afferent nerve fibres. In none of the experiments quoted above can the results of section of the depressor nerve be attributed to anything but interruption of baroreceptor afferent nerve fibres.

The effects of occlusion of the left carotid artery (the right having been tied previously) of the experiments on raising the pressure in the carotid sinus, and of the Valsalva manoeuvre all provide direct evidence that the

threshold of the baroreceptors is within the normal pressure range of the arterial pulse in a new-born rabbit. Once again, however, the changes in heart rate were very small.

We now have to consider how it came about that Bauer (1939) and Barcroft (1946) came to the conclusion that the baroreceptor reflexes were not normally in action in the new-born rabbit. With the apparatus then available Bauer found it difficult to record the arterial pressure in new-born rabbits, and relied mainly upon the heart rate as an index of changes in the cardiovascular system. Barcroft wrote: 'If the depressor reflex is present, ligature of the depressor will quicken the heart'. This may be true of the adult, but not of the new-born rabbit, in which there is a significant rise in blood pressure on section of the depressor nerves, but a negligible change in heart rate. Bauer's paper shows falls of heart rate on stimulation of the depressor or carotid sinus nerve, of which the greatest is 33%. This is just a little more than the most I saw (29%) in forty rabbits 0–15 days old on maximal stimulation, but the *mean* fall in heart rate was only $9{\cdot}5 \pm 2{\cdot}7$ (S.E.)%. The change in blood pressure was considerably greater. All Bauer's observations were made under urethane anaesthesia, and, as compared with pentobarbitone, this anaesthetic very much reduced the size of the response in the first 5 days after birth. Neither of these considerations alter the fact that Bauer observed a difference as between rabbits 21 days and 34–42 days old when the depressor nerves were cut during asphyxia. In the older rabbits there was an immediate increase in heart rate, which did not occur in the younger. However, his other observations show that the rise in blood pressure during asphyxia was greater in older than in young rabbits, so that the depressor afferent activity would have been greater in the older. The fact that cutting the depressor nerves during asphyxia had no demonstrable action on the heart rate in the young rabbits does not necessarily mean that the depressor reflex was not in action either in asphyxia or in the normal animal.

Finally, there are other problems which will require further investigation. During the first 5 days of life in rabbits the heart rate increases (Fig. 1). After several weeks of life the heart rate begins to decrease according to Bauer (1939). Throughout the whole of this period of time blood pressure is gradually rising. Bauer attributed the secondary fall in heart rate to the rise in blood pressure above the threshold of the depressor and carotid sinus reflexes. If these reflexes are active at birth, this explanation is no longer satisfactory. Another question concerns the sensitivity of the baroreceptors to transmural pressure. The evidence at present available (as in Fig. 10) would be consistent with the view that, with increasing age, the threshold mean arterial pressure required to excite

receptors increases. But there is no certainty that some unconscious selection was not at work (e.g. that those fibres which survived the process of dissection had different characteristics from the mean). The problem as to whether barosensory receptors change in sensitivity with increasing age, and so with increasing arterial pressure, may have to be examined in other ways.

SUMMARY

1. The aortic depressor and carotid sinus baroreceptor reflexes were studied in new-born rabbits.

2. Electrical stimulation of the central end of one depressor nerve caused a 17 % (mean) fall in blood pressure and a small reduction in heart rate. When one carotid sinus nerve was stimulated there was either a rise in blood pressure or a fall according to the strength of the stimulus. Stimulation of the distal vagus caused a slowing of the heart (65 % mean) and a reduction in blood pressure (45 % mean).

3. When the depressor and carotid sinus nerves were cut there was a rise of blood pressure but very little change in heart rate. Occlusion of the carotid arteries caused a 19 % (mean) increase in blood pressure. This was much reduced by cutting the carotid sinus nerve.

4. Raising the pressure in one carotid sinus isolated from the rest of the circulation caused a fall in systemic blood pressure. Pulsatile pressures with mean values of 30 or 40 mm Hg produced a significantly greater response than non-pulsatile pressures. There was a positive correlation between the rise in carotid-sinus pressure and the percentage fall in systemic arterial blood pressure.

5. As compared with pentobarbitone, urethane anaesthesia reduced or abolished baroreceptor reflex responses in rabbits less than 6 days old.

6. Single and multifibre preparations of the depressor and carotid sinus nerves demonstrated activity synchronous with the pulse wave during the first few days of life.

7. In the rabbit, during the first week after birth, the blood pressure is relatively low; nevertheless it was concluded that the baroreceptor reflexes are functional and maintain tonic activity.

I wish to express my deep appreciation to Dr G. S. Dawes for his advice and encouragement throughout this work, and to Mr M. Richings and Mr G. Smith for their technical assistance. This work was done during the tenure of a Life Insurance Medical Research Fellowship.

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THE RECOVERY OF RESPONSIVENESS OF THE SENSORY SYNAPSES IN THE LATERAL GENICULATE NUCLEUS

BY P. O. BISHOP AND R. DAVIS*

From the Brain Research Unit, Department of Physiology, University of Sydney, Sydney, Australia

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The normal ganglion-cell discharge from the retina consists of a brief high-frequency burst of impulses. The short refractory period and prolonged phase of supernormality in the recovery cycle of the optic nerve and tract fibres are properties well suited to handle such a discharge (Bishop, Jeremy & Lance, 1953). The ganglion-cell discharge may, however, undergo important modification in the lateral geniculate nucleus before relay to the cerebral cortex. One of the most important factors determining such a modification is the nature of the recovery cycle of the synapses and neurones in the nucleus. A detailed account of the recovery of the geniculate synapse from the refractory state has already been published (Bishop & Evans, 1956). Recovery from refractoriness in this nucleus is principally determined by the rate at which normal conduction ability returns in the presynaptic fibres. Slowed conduction in relatively refractory axons means that the absolutely refractory period of the synapse is greater the longer the effective presynaptic pathway. An upper value of 0·85 msec was set for the absolutely refractory period, with conditioning and testing stimuli applied to the presynaptic pathway (optic tract) about 10 mm from the lateral geniculate nucleus. The geniculate neurones were always capable of responding as soon as conduction became possible in optic tract fibres. By stimulation of the optic nerve and with spike height as an index, the relatively refractory periods of the presynaptic and post-synaptic spikes recorded in the lateral geniculate nucleus were 1·7 and 1·6 msec, respectively. Supernormal excitability on the part of the geniculate neurones commences while the optic nerve fibres are still relatively refractory.

It is the aim of the present paper to describe the stages of the recovery cycle from refractoriness up till 5 sec after the discharge of the geniculate neurones. A re-examination of the results of Bishop & Evans (1956) shows that the early phase of supernormality referred to above extends from about

* Ophthalmic Research Institute of Australia Fellow.

0·95 to 1·65 msec. The results given below show that the recovery cycle which follows this early stage then shows alternating periods of subnormal and supernormal responsiveness, each of increasing duration. These phases are examined in detail. Recovery after brief repetitive stimulation will be described in a subsequent paper together with an analysis of the relationship between recovery and the after-potentials of the geniculate neurones. The earlier work of G. H. Bishop & O'Leary (1940), Marshall & Talbot (1940, 1941, 1942), Talbot & Marshall (1941) and Marshall (1949) on the recovery of responsiveness of the geniculate neurones will be discussed in relation to our findings.

Understanding of the fundamental properties of the spinal motoneurones and of the synapses associated with them is now much more complete than that of any other neurone type in the central nervous system. It is clear, however, that other neurone types and synaptic centres in the central nervous system have properties some of which diverge widely from those characteristic of the spinal motoneurone. For this reason it is important that attention should be directed towards the study of a variety of synaptic centres. The earlier paper of Bishop & Evans (1956) and the present study provide information relating to the recovery properties of sensory neurones and their associated synapses.

METHODS

Adult cats were used in all experiments, and were anaesthetized with intraperitoneal allobarbitone (Dial, Ciba, 0·5 ml./kg.). If necessary small amounts (totalling 0·3 ml.) of sodium pentobarbitone (Sagatal, May and Baker) were added intravenously. The cat's body temperature, read with a rectal thermometer, was controlled within normal limits (38–39° C) with the aid of an electric heating blanket. The general methods used have been adequately described in previous communications from this laboratory (cf. particularly Bishop, Jeremy & Lance, 1953). The eyeball was resected and the optic nerve prepared for electrical stimulation by being suspended clear of orbital tissue. The response of the opposite lateral geniculate body was recorded by means of a stereotaxically directed steel micro-electrode introduced down through the intact cerebral cortex. The recording position was determined by electrolytically depositing iron from the tip of the micro-electrode by means of a current of 30–40 μA flowing for 15 sec; the brain was subsequently perfused, first with normal saline and then with 10% formalin saline containing 1 g/100 ml. of potassium ferrocyanide. The blue spot at the recording site was found in serial histological sections after paraffin embedding.

RESULTS

The notation used for referring to the principal structures contributing to the wave form of the geniculate response following optic nerve stimulation is standard for this laboratory (cf. particularly Bishop & McLeod, 1954; Bishop & Evans, 1956). Ordinarily we use the term 'contralateral t_1' to designate the group of tract fibres of larger diameter that have come from the contralateral optic nerve. Since only crossed fibres have been used in this investigation the term 't_1' will be sufficient, and also with respect to the term 'r_1' which

refers to the corresponding post-synaptic propagated discharge of the geniculate neurones. Similarly the terms 't_2' and 'r_2' will be used to refer respectively to the presynaptic and post-synaptic responses in the lateral geniculate nucleus associated with activity in the group of optic tract fibres of smaller diameter (Fig. 1, C.R.). The analysis of the wave form of the geniculate response and the method used for measuring it have been given in earlier publications (cf. particularly Bishop & Evans, 1956).

The curve of recovery of responsiveness of the geniculate synapses following transmission was determined by applying a test volley to the synapses and progressively increasing the interval between conditioning and testing shocks

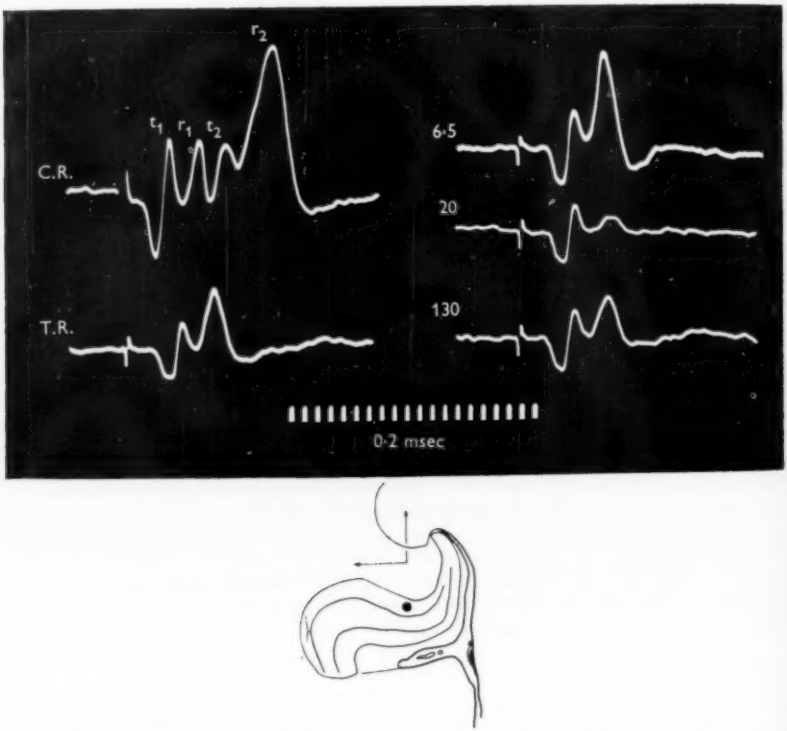

Fig 1. Recovery of responsiveness of geniculate synapses following a maximal conditioning shock applied to the contralateral optic nerve. C.R., conditioning response; t_1, t_2, presynaptic spikes; r_1, r_2, corresponding post-synaptic spikes. T.R., unconditioned test response, 47% maximal for t_1. Remaining traces show conditioned test responses at shock intervals indicated in msec. Recording site was close to filled circle in outline drawing of parasagittal section of lateral geniculate nucleus; arrows indicate horizontal and vertical Horsley–Clarke planes. For details see text.

in the usual way. In carrying out this procedure and in assessing the results obtained a variety of factors had to be taken into consideration. It will be convenient to discuss these factors first, before presenting the curve of recovery of responsiveness. While all these factors are of importance in this context, some are of technical interest only, whereas others also merit consideration because of the clues they provide to the organization of the lateral geniculate nucleus. The detailed description of our results from the latter point of view will be reserved for a later publication.

Factors associated with the conditioning response

In order to test recovery processes it is important to ensure that the testing volley should only reach geniculate neurones that have been discharged by the conditioning volley. The conditioning shock was therefore made supramaximal for all the fibres in the optic nerve. It remained possible, however, that a maximal optic nerve volley might still have associated with it a fringe of subliminally excited geniculate neurones that could be brought to discharge only by a suitably timed second volley. Bishop & Evans (1956) have already discussed this problem. From an analysis of the random fluctuations in the amplitude of the geniculate response to a constant afferent volley, they concluded that the subliminal fringe associated with a smaller presynaptic volley is completely discharged by a larger independent volley. In other words, as the unconditioned presynaptic volley grows in amplitude with increasing shock strength the geniculate cells are progressively brought into the discharge with no 'islands' of subliminal fringe excitation remaining. Several other lines of evidence lend strong support for this view, namely:

The relationship between the amplitudes of the t_1 and r_1 spikes, normally and with conditioning. Curve (*c*) of Fig. 2 shows the normal unconditioned t_1/r_1 relationship and the other curves show the relationship at the indicated times (4·2, 10·5 and 20·5 msec) after a conditioning volley maximal for t_1 and t_2. It will be seen that, at the 4·2 msec interval, the increase in amplitude of the r_1 spike due to supernormal excitability on the part of the geniculate neurones is independent of the amplitude of the r_1 response over practically the whole range of the afferent bombardment. The increase in the r_1 spike due to supernormal excitability is a measure of the size of the available subliminal fringe, since the latter determines the maximum facilitation that can occur with a test volley whatever the means used to obtain the facilitation. The fact that the amount of the facilitation is constant and independent of the amplitude of the r_1 spike indicates not only that the size of the subliminal fringe is constant, but also that the fringe associated with a smaller presynaptic volley is completely discharged by a larger independent volley.

At an interval of 10·5 msec after the conditioning response (Fig. 2) the r_1 spike is reduced because the recovery cycle of the geniculate synapses has

now entered a prolonged phase of depression. Again, the amount of the depression is constant and independent of the amplitude of the r_1 spike. At an interval of 20·5 msec the depression of the r_1 spike has greatly increased being about maximal at this time (Fig. 3*d*). In all the curves in Fig. 2 the reduction in amplitude of the r_1 spike as the t_1 volley reaches a maximum is due to summation of the r_1 negativity with the prodromal positivity of the t_2 spike.

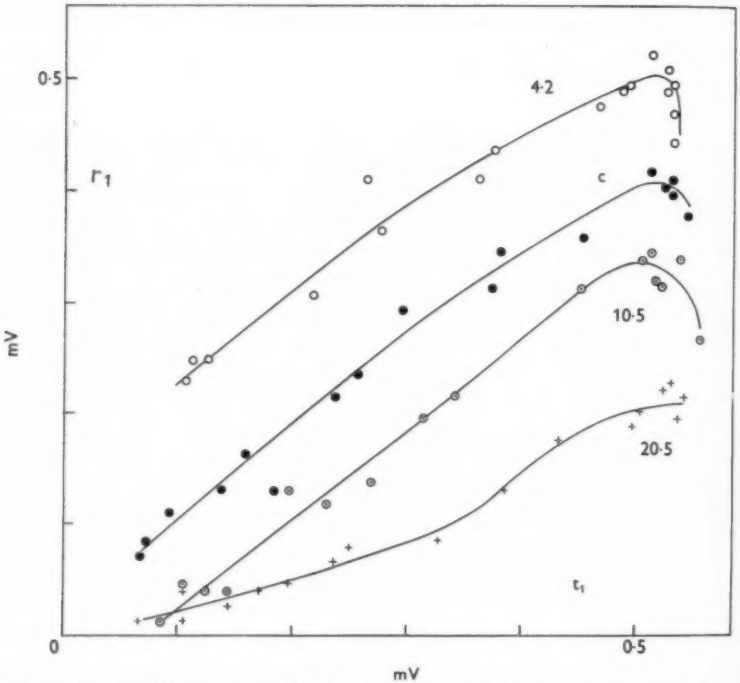

Fig. 2. Relationship between amplitude of presynaptic spike (t_1) and post-synaptic spike (r_1) in response to increasing stimulating shocks applied to the contralateral optic nerve. (C), normal control curve. Remaining curves show the relationship at times indicated in msec following a conditioning shock which was maximal for t_1 and t_2. Abscissae (t_1) and ordinates (r_1) are given in millivolts. Recording site indicated by filled circle in outline drawing in Fig. 1. For details see text.

For this reason the plotting of the curves was stopped just short of a maximal t_1 volley. The change in slope of the curve at the 20·5 msec interval, when the amplitude of the t_1 volley falls below about 50% maximal, is due to the fact that the post-synaptic propagated discharge is now failing almost completely and the amplitude of the response comes to be determined very largely by the non-propagated synaptic potential.

Maximal conditioning and testing shocks. By using maximal conditioning and testing volleys the possibility of a 'supra-maximal' geniculate response was sought at nine recording sites in five preparations. Shock intervals between 3 and 5 msec were chosen to correspond with the peak of the late phase of increased synaptic responsiveness (see below). It is difficult to study the early phase of facilitation when using a maximal conditioning shock because the later stages of the conditioning response in the lateral geniculate nucleus interferes with the recording of the testing volley. The detailed analysis of these results will be published elsewhere. For our present purposes it will be sufficient to say that there was no indication that a fringe of subliminally excited neurones was available to the t_2 test volley under these circumstances; on eight of the nine occasions the r_2 spike was substantially reduced (by 20–30%) and one one occasion it was unchanged. The behaviour of the r_1 spike was somewhat more complicated; it was usually unchanged (five occasions) once it was slightly reduced (to 85%) and on another occasion it could not be measured because of the superimposition of the r_1 and t_2 spikes. On the remaining two occasions the r_1 response was clearly increased in amplitude (125 and 142% respectively, normal = 100%), the r_2 response being then reduced by an amount about the same as that by which the r_1 response was facilitated. Our explanation for the latter finding, based on a detailed analysis of the strength–response curves and on single unit recording (to be published elsewhere) is that there are occasional cells in the nucleus that are innervated both by t_1 and t_2 afferent fibres. In the present instance these cells were normally discharged only by the t_2 fibres, being subliminally excited by t_1 fibres. During the supernormal phase of recovery from discharge, however, the cells were then fired by the t_1 volley and were refractory to the t_2 volley. The important observation for the present paper is that, although there may be occasional cells in the nucleus that lie in the subliminal fringe of a maximal t_1 volley, they are nevertheless discharged by a volley maximal for t_2.

Post-tetanic potentiation. A study of the phenomenon of post-tetanic potentiation provides information regarding the size of the subliminal fringe about active geniculate neurones. With supramaximal conditioning and testing shocks the mean post-tetanic potentiation of the r_1 spike in five experiments was only 103% (normal = 100%); in only one experiment was there a significant increase (Bishop, Burke & Hayhow, 1959). It is interesting to note that with a submaximal testing shock the post-tetanic potentiation was 114%, which is about the same as the post-discharge supernormal responsiveness reported in this paper. This finding presumably indicates that both phenomena are limited in the same way by the available subliminal fringe.

All the above evidence indicates that a maximal optic nerve volley does not leave a fringe of subliminally excited neurones. A testing volley under these

circumstances must therefore only reach synapses and neurones that have been discharged by the conditioning volley; i.e. it tests post-discharge recovery processes and not subliminal excitation.

Method used for expressing results

The fact that the amount of the facilitation or depression is constant over almost the whole range of test volley size has another important consequence: it provides a way of comparing the degree of facilitation or depression from one experiment to another and so generalizing the results obtained. In this paper changes in post-synaptic responsiveness, both in the direction of facilitation and depression, are expressed as a percentage of a fixed r_1 amplitude, namely, the r_1 spike produced by an afferent volley which is 50% maximal for t_1. The latter procedure was adopted because it is easy to determine the maximal amplitude of the t_1 spike, whereas the r_1 spike is affected by the appearance of the t_2 response. The relationship between the amplitudes of the t_1 and r_1 spikes was plotted in every experiment and the amplitude of the r_1 spike produced by a 50% maximal t_1 spike could be read off from this graph (cf. Bishop & Evans, 1956).

Factors associated with the test response

Many of the factors which have to be considered in relation to the test response have already been discussed in an earlier paper (Bishop & Evans, 1956). These include the selection of the recording site, the quantitative analysis of the wave form of the geniculate response, spontaneous fluctuations in the excitability of the geniculate neurones and effects of changes in the depth of the anaesthesia. Two factors constitute a special hazard, namely, the movement of the brain with respect to the micro-electrode and changes in the threshold for stimulation of the optic nerve. With regard to the former, when a suitable recording site was found it was usual to wait about half an hour before commencing the series of recordings, to allow the geniculate tissue to adapt itself, mechanically and biologically, to the presence of the micro-electrode. Any movement is usually indicated by changes in the relative amplitudes of the t_1 and r_1 spikes. Plots of the t_1/r_1 relationship both before and after a particular recording series were usually carried out and compared (cf. Bishop & Evans, 1956). The main factor altering the stimulus threshold of the optic nerve was the shunting effect of the collection of tissue fluid. Usually the nerve can be suspended clear of orbital tissue in such a way that this is not a problem and careful swabbing every 1–2 hr, or the installation of drainage, usually ensures that the threshold remains almost constant during the recording series. A large number of unconditioned test responses recorded throughout the recovery cycles provided a check on the constancy of the recording conditions. The interval between each sequence of conditioning

and testing stimuli (5–10 sec) was chosen to avoid as far as possible any cumulative changes in excitability. The early phases of recovery, covering supernormality and early subnormality, were usually recorded twice for each cycle.

Presynaptic excitability changes

Using antidromic volleys in the optic nerve, Bishop, Jeremy & Lance (1953) have already studied the changes in the excitability of the optic tract fibres during recovery from discharge. By recording the optic tract volleys in the lateral geniculate nucleus, however, it is possible to obtain information about the properties of the fibre terminals. Details of the recovery of the fibre terminals from the refractory state have already been published (Bishop & Evans, 1956) and the later stages of the recovery process are reported here (see Table 1 and 2; Figs. 3*a* and 5*a*). The data given in Tables 1 and 2 were obtained from eleven preparations in which a total of nineteen recovery cycles were analysed. Supernormality commences about 1·7 msec after discharge,

TABLE 1

t_1 amplitude (% of maximal)	Number of cycles	Mean supernormality (%)
30–39	8	179
40–49	6	159
50–59	3	139
60–69	2	134

reaches a peak at 4·6 msec and terminates at about 34 msec. Subnormality does not normally occur following a single conditioning volley. The time course of the phase of supernormality is very similar to that reported by Bishop Jeremy & Lance (1953) using antidromic stimulation and, like the r_1 spike, we found it to be independent of the amplitude of the t_1 volley. By contrast with the antidromic results, however, the amplitude of the response on the part of the fibre terminals showed a much greater increase during supernormality, having an average maximum of 151% for a volley which was 50% maximal for t_1. Between 110 and 120% was the value obtained by stimulating the optic tract and recording from the optic nerve. It is difficult, however, to make a valid comparison between these results because of the difficulty in obtaining maximal stimulation of the whole optic tract by means of buried electrodes. It is probable, however, that, as far as supernormality is concerned, the fibre terminals do not differ from the portion of the axon in the optic tract. In one experiment bipole electrodes were placed in the optic tract between the chiasma and the nucleus. The recovery cycles of the tract fibres at that site were obtained by recording the antidromic volley from the nerve and the reverse procedure gave the recovery cycle of the optic nerve fibres. The maximum supernormality in the two instances was 118 and 153%, respectively, the orthodromic test volley in the latter instance being 49%

maximal for t_1. Thus if different parts of the axon vary with respect to the degree of supernormality it is the optic nerve on the one hand which differs from both the optic tract and fibre terminals on the other. This may, however, be a result of the preparation and general treatment of the optic nerve rather than a true physiological difference.

It has been pointed out above that the amount of the facilitation of the r_1 spike during supernormality is constant with increasing volley size so that the percentage facilitation progressively declines. The same is true of the t_1 response (see Table 2) and this fact again enables us to compare the supernormality found in one experiment with that found in another by expressing the amount as a percentage of the amplitude of the tract spike which was 50% maximal for t_1. In order to test the validity of this method of comparison the mean supernormality found in seven recovery cycles in which the amplitude of the t_1 volley was close to 50% maximal (range 47–54, mean 50%) was compared with the mean supernormality in the remaining twelve recovery cycles, in which the amplitude of the t_1 volley fell outside this range (i.e. 32–62%). The former group has a mean actual supernormality of 149% while the latter had a mean calculated supernormality of 152%. The above details are interesting in relation to the organization of the lateral geniculate nucleus because they indicate that any given small sample of geniculate neurones is innervated by afferents having a wide and fairly smooth range of fibre diameters. Other evidence is available which points in the same direction (Bishop, 1959).

Despite the fact that test shocks of constant strength are applied to the optic nerve, it is obvious that the corresponding volleys applied to the geniculate synapses undergo marked fluctuations during the recovery cycle. Marshall (1949) attempted to obtain constant test volleys by repeated adjustment of the intensity of the conditioning shock. Unfortunately, under the experimental conditions it is very difficult to do this with any precision. In this study corrections were made after the experiment by using the graph of the unconditioned t_1/r_1 relationship to obtain the r_1 spike that would be expected for any given amplitude of t_1 volley. Regarding the amplitude of the r_1 spike obtained in this way as the unconditioned post-synaptic spike, the amount of the facilitation or depression was then found by subtraction from the conditioned spike actually recorded. Plots of the t_1/r_1 relationship were carried out before and after the recording of a particular recovery cycle and it was important to determine that, during the cycle itself, the regularly recorded unconditioned test response did fall on this t_1/r_1 line. The amounts of facilitation or depression were then expressed as a percentage of the unconditioned r_1 spike produced by a presynaptic volley which was 50% maximal for t_1.

Both methods of correction mentioned above assume that the changes in

amplitude of the t_1 volley are due solely to fibres entering or leaving the response, the single spike in the response remaining unchanged. It is possible, however, that general and regional fluctuations, both of the membrane potential and of the time relations of the impulse, could lead to alterations in the amplitude of the t_1 spike without involving an increase or decrease of the number of fibres activated. That the latter effects can be neglected for the present purpose is indicated by the fact that, on nine occasions, the conditioned t_1 response was never increased and was not reduced by more than a few per cent when maximal conditioning and testing shocks were used. Any change in amplitude that occurs when submaximal shocks are used is, therefore, due almost entirely to fibres entering or leaving the response. As was pointed out above, the results can be expressed in a way that is independent of changes in the amplitude of the unconditioned r_1 response.

The early supernormal phases

The results of a typical experiment are shown in Figs. 1, 2 and 3; these were all obtained from the same preparation. The tip of the micro-electrode was located in layer A (Thuma, 1928) at approximately the anteroposterior and mediolateral mid point of the lateral geniculate nucleus (1·5 mm from the medial edge and 2·8 mm from the anterior pole; Fig. 1). The traces shown in Fig. 1 were selected from those used in the preparation of Fig. 3*a*, *c* and *d*. The conditioning response (C.R. Fig. 1) was maximal for t_1 and t_2 while the test response (T.R.) was 47 % maximal for t_1. The t_1/r_1 graph shown in Fig. 3*b* was obtained immediately before the recording of the recovery cycle (Fig. 3*a*, *c* and *d*). The t_1, r_1 values of the mean unconditioned test response obtained during the recovery cycle are indicated by the arrow (Fig. 3*b*). Some hours later the traces used in the preparation of Fig. 2 were recorded but, in the interval, a very slight movement of the micro-electrode had occurred so that graph *C* of Fig. 2 is not comparable to Fig. 3*b*. The recording site was then labelled by deposition of iron from the micro-electrode (Fig. 1).

With a maximal conditioning volley (C.R. Fig. 1) the presence of the r_2 spike made about 2·5 msec the least shock interval at which a measurement of the test response could be made. Graph (*a*) of Fig. 3 shows the time course of the supernormality of the presynaptic fibres. Graph (*c*) shows the time course of the recovery cycle of the post-synaptic spike, uncorrected for changes in t_1, from about 2·5 msec until recovery is complete. Unconditioned test responses were recorded before every conditioning–testing sequence and the changes in the conditioned r_1 spike have been expressed in graph (*c*) simply as a percentage of the corresponding unconditioned spike. In graph (*d*) the measurements of the conditioned r_1 spike have been corrected for changes in the t_1 volley and then plotted as a percentage of the r_1 spike produced by a presynaptic volley that was 50 % maximal for t_1. When plotting commenced,

supernormality was already present and persisted for a shock interval of 8·6 msec.

Of the total of eighteen recovery cycles recorded from ten cats, fourteen clearly showed this late phase of supernormality, one returned only to normal and the remaining three approached normality before the onset of the phase of deep subnormality. Taking all these experiments into account the peak of

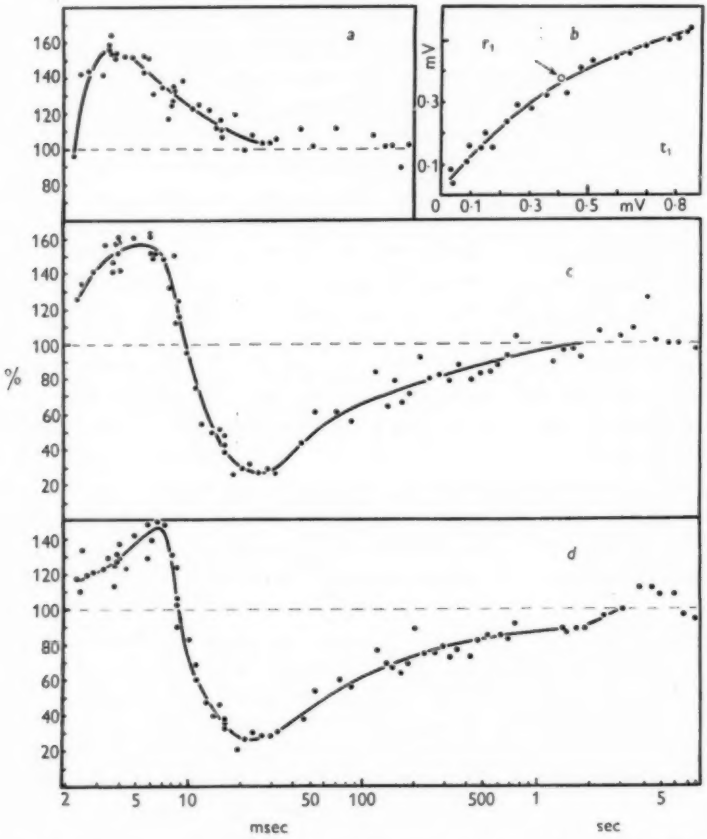

Fig. 3. Curves of recovery of responsiveness of geniculate synapses following a maximal conditioning shock applied to the contralateral optic nerve. Responses in Fig. 1 were selected from those used for above curves. Curve (*a*), recovery of presynaptic spike (t_1). Curve (*b*), relationship between amplitudes of presynaptic and post-synaptic spikes in response to increasing afferent stimulation; open circle indicated by arrow shows mean unconditioned test response obtained during recovery cycle. Curves (*c*) and (*d*), recovery of post-synaptic spike, uncorrected (*c*) and corrected (*d*) for presynaptic excitability changes. Abscissæ (log-scale) are common to graphs *a*, *c* and *d*. For details see text.

supernormality had a mean corrected value of 113% at a shock interval of 4·7 msec. Details of both the corrected and uncorrected recovery cycles are given in Table 2. The fact that conduction velocity recovered to normal at a shock interval of about 1 msec (cf. Graham & Lorente de Nó, 1938) means that the shock intervals given in the table can be translated directly into response intervals without significant error. It will be seen that, owing to the prolonged increase in the t_1 volley, the supernormality in the uncorrected recovery cycles had a mean value of 181% and persisted for a shock interval of 9·9 msec.

Shock intervals between recovery from absolute refractoriness (about 0·8 msec) up till 2·5 msec cannot be studied by using a maximal conditioning volley (see above). Bishop & Evans (1956) have, however, studied this interval by using a conditioning volley that was usually just submaximal for t_1. They pointed out that supernormality on the part of the r_1 spike began before the return to normal of the t_1 spike at about 1·7 msec. There was clearly an early phase of supernormality that could not be accounted for by changes in the size of the t_1 volley. Using Bishop & Evans's data we have now defined this phase of supernormality by means of a systematic correction for changes in the t_1 volley (Fig. 4). Six preparations provided data which were suitable for this detailed analysis and the results are set out in Table 2. Supernormality commenced at 0·95 msec (mean shock interval), reached a peak of 114% at 1·14 msec, and terminated at 1·66 msec. An early peak of supernormality round about 1 msec was present in all the six preparations examined. Unfortunately, with the smaller conditioning volley the longest shock intervals used by Bishop & Evans were generally less than 3 msec, so that there was only a relatively small overlap between their recovery cycles and those reported in this paper.

Figure 5*A* and *B* shows the corresponding presynaptic and post-synaptic recovery cycles, respectively, with shock intervals up to 10 msec, the phases being recorded successively at the same site in the nucleus. The two phases of supernormality of the r_1 spike are seen to be separated by a phase of relative subnormality at about 2 msec. In recording this cycle the conditioning shock was just submaximal for t_1 with shock intervals up to 2 msec, and thereafter it was made maximal for t_1 and t_2. The early phase of supernormality ends at about the same time as the conditioned t_1 and r_1 spikes regain their unconditioned amplitude (cf. Bishop & Evans, 1956). That the two phases of supernormality can be distinguished may, therefore, be due to the fact that the conditioned test volley is increasing rapidly in amplitude during the first 2–3 msec, whereas it remains relatively constant during the subsequent 5 msec (Fig. 5*A*). The early phase is, therefore, one of relative supernormality only, whereas the later phase is one of true supernormality because both the conditioned t_1 and r_1 spikes are actually larger than normal.

TABLE 2

		Absolutely refractory period (msec)	Supernormality: Phase	Onset (msec)	Peak time (msec)	Peak supernormality (%)	Termination (msec)
t_1	Antidromic*	0·47	—	(2–3)	(4–6)	(110–120)	33 (20–45)
	Orthodromic	0·81 (0·7–0·92)†	—	1·7 (1·4–2·0)†	4·6 (3·3–6·5)	151 (132–180)§	34 (22–70)
r_1 (corrected)	—	< 0·85†	Early phase‖	0·95 (0·94–0·96)	1·14 (0·83–1·42)	114 (102–126)§	1·66 (1·05–2·2)
			Late phase	–(< 2·0–4·3)	4·7 (2·4–7·0)	113 (77–146)§	6·7 (3·6–11·3)

		Absolutely refractory period (msec)	Onset, early phase	Peak time, late phase	Peak supernormality late phase (%)	Termination late phase
r_1 (uncorrected)	—	0·81 (0·70–0·92)†	1·6 (1·35–1·85)†	4·8 (2·7–6·0)	181 (128–292)	9·9 (6·7–13·8)

The values given are mean values, the range of variation being given in brackets.

* Bishop, Jeremy & Lance (1953). † Bishop & Evans (1956). § Expressed as % of amplitude (t_1 or r_1) when t_1 was 50% maximal.

‖ Based on data from Bishop & Evans (1956).

TABLE 3

	Subnormal phase: Uncorrected maximum subnormality	Corrected: Maximum subnormality	60	80	Change of slope	90	100	Terminal supernormality, Corrected: Peak	Termination
r_1 (% recovery)	44 (31–68)	32 (20–44)			87 (75–100)			114 (8–30)	100
Shock interval (msec)	24 (16–30)	19 (15–26)	83 (70–90)	170 (130–230)	220 (170–250)	1050 (500–1800)	2 sec* (1·4–3·0)	3·8 sec* (2–5·5)	5 sec* (4·5–6)

The values given are mean values, the range of variation being given in brackets. Shock intervals are in milliseconds except where indicated.*

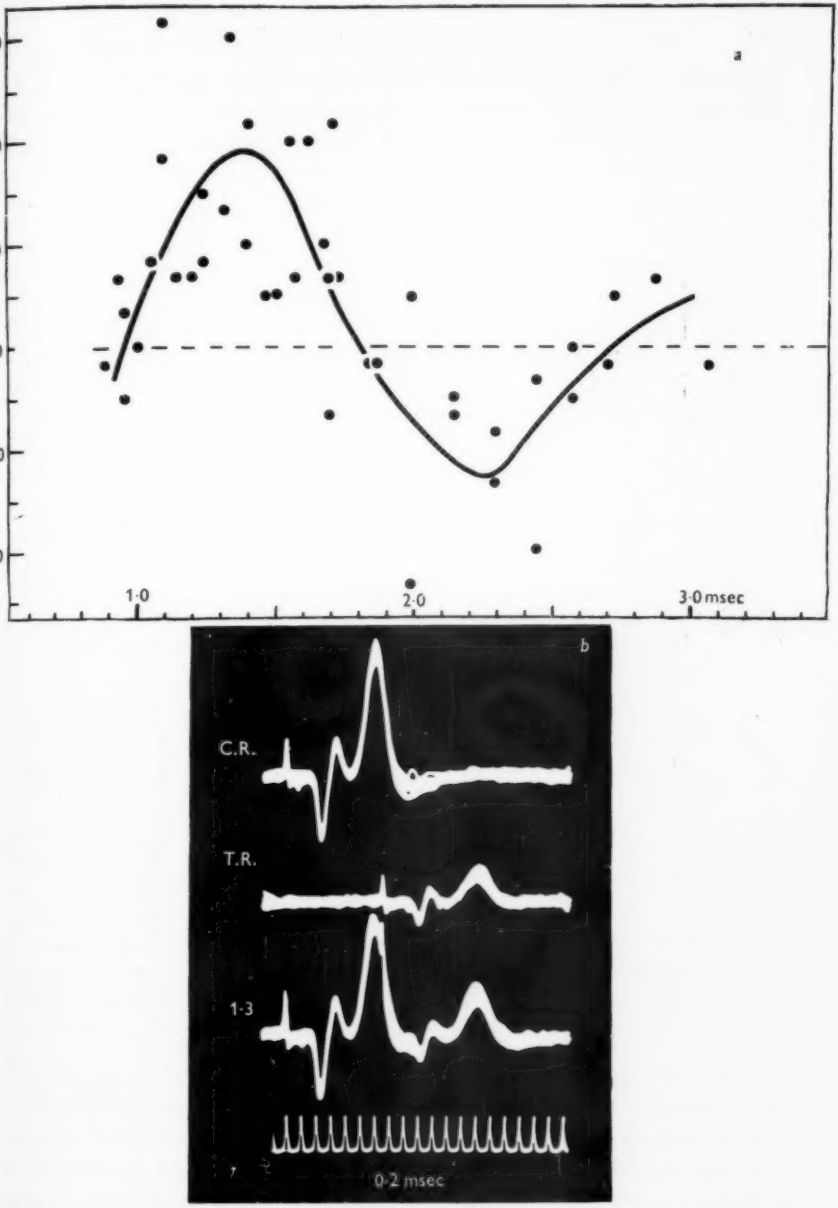

Fig. 4. Recovery of responsiveness of geniculate synapses during first 3 msec after a conditioning shock, 90% maximal for t_1, applied to contralateral optic nerve. Test stimulus 31% maximal for t_1. (*a*) Post-synaptic spike recovery curve corrected for changes in the t_1 spike due to relative refractoriness and subsequent supernormality. (*b*) Superimposed geniculate responses (10 traces each). C.R., conditioning response, T.R., test response, unconditioned and conditioned at a shock interval of 1·3 msec.

The values given are mean values, the range of variation being given in brackets. Shock intervals are in milliseconds except where indicated.*

(msec)

A further complication is introduced by reason of the fact that, during the early phase, the conditioning shock was necessarily always only maximal for t_1 (or just submaximal) whereas, with one exception, it was always made maximal for both t_1 and t_2 throughout the remainder of the recovery cycle.

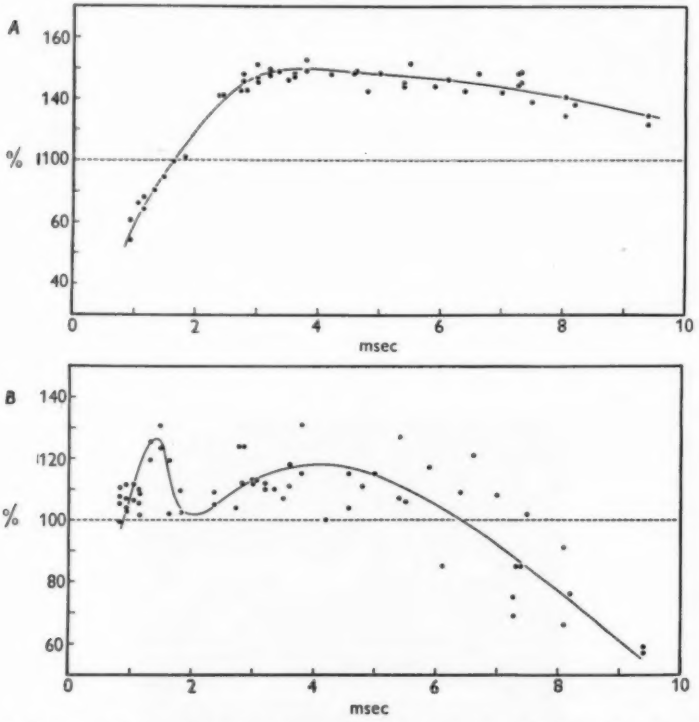

Fig. 5. Recovery of responsiveness of presynaptic (*A*) and post-synaptic (*B*) spikes. Conditioning shock applied to contralateral optic nerve just submaximal for t_1 at shock intervals up to 2 msec and thereafter maximal for both t_1 and t_2. Post-synaptic recovery curve corrected for presynaptic excitability changes. For details see text.

On the one occasion when a conditioning shock maximal for t_1 only was used throughout the whole recovery cycle there was no clear separation between the two phases. On another occasion, however, when the wave form of the conditioning response (maximal for both t_1 and t_2) allowed measurements to be made at shock intervals as early as 2 msec, there was a clear indication of an intervening phase of relative subnormality.

It is interesting to note that in the case of unit repetitive firing in the lateral geniculate nucleus the least spike interval is about 0·8 msec (unpublished

observations). The preferred intervals for repetitive firing, as indicated by the response obtained from cell populations (Bishop, Jeremy & McLeod, 1953), may also be a function of the phases of supernormality described above.

The subnormal phase

Immediately following the late phase of supernormality, at a shock interval of about 6·7 msec, the curve of recovery of responsiveness crosses sharply to a phase of deep and prolonged subnormality (Fig. 3*d*). Mean values for the various portions of the curve of recovery from depression are given in Table 3. Maximum depression (32%) is reached at a shock interval of 19 msec, and thereafter the r_1 spike recovers, at first rapidly and then increasingly slowly. In all the curves there is evidence of a two-stage recovery process with a change of slope or 'knee' occurring at a shock interval of about 220 msec, when the response is about 87% recovered. Though it is not very marked, this change of slope can be seen in Fig. 3*d*. The second stage of recovery takes about a further 1·8 sec to overcome the remaining 13% of depression.

In the four recovery cycles in which shock intervals much beyond 2 sec were used there was evidence of a further phase of supernormal responsiveness having a peak at about 3·8 sec and ending at about 5 sec (Fig. 3*d*). Further work will have to be done in order to describe this late phase more adequately. The possibility of further fluctuations in responsiveness beyond about 5 sec was not examined.

Electrode position in the lateral geniculate nucleus did not seem to be an important factor in determining the form and time course of the recovery cycle. In fact recovery cycles recorded at different positions in the one preparation were closely similar. Depth of anaesthesia is probably a much more important variable. Animals were always as lightly anaesthetized as possible but in one preparation the effect of the intravenous administration of 0·5 ml. of Sagatal was studied. The drug caused a reduction in the peak supernormality of the t_1 spike and at the same time, an increase in the peak supernormality of the r_1 spike. It is unlikely that this increase in r_1 supernormality is due to a change in the available subliminal fringe because the t_1/r_1 graph remained unaltered. Further work will have to be done to elucidate the effect of the anaesthetic.

DISCUSSION

The idea that both the parts of the neurone involved in synaptic transmission, and the transmission process as a whole, would be found to have properties which would resemble, qualitatively at least, those of the axon has long been an obvious working hypothesis (cf. Gasser, 1939). It was early found that the synapses in sympathetic ganglia have a classical recovery cycle which involves an early phase of supernormality and a late phase of depression (Eccles, 1935;

Lloyd, 1939*b*) and a good correlation was obtained between the orthodromic recovery cycle and the ganglion after-potentials (Lloyd, 1939*a*).

By stimulating the posterior longitudinal bundle and adjacent tracts in the brain stem and recording the internal rectus muscle action potential Lorente de Nó (1935) estimated the absolutely refractory period of the oculomotoneurone synapses to be 0·6 msec. A maximal muscle action potential was taken as an indication that the oculomotoneurone pool was being maximally discharged by the presynaptic volley. Bishop & Evans (1956) have already pointed out that an important correction is necessary by reason of slowed conduction in the relatively refractory presynaptic fibres. Another difficulty, however, remains. Lorente de Nó's Fig. 2 shows that at a shock interval of 1·45 msec the test muscle response was approximately recovered to normal. Nevertheless, the recovery cycle of oculomotoneurones studied by Lorente de Nó & Graham (1938) with antidromic conditioning and orthodromic testing volleys showed no evidence of this early phase of rapid recovery to near normality. Instead, absolute refractoriness was followed only by a prolonged phase of gradually lessening depression. Subsequent investigations of the recovery cycle of spinal motoneurones with the same method of conditioning and testing (Brooks, Downman & Eccles, 1950*a*; Lloyd, 1951) have revealed absolute refractoriness to be succeeded directly by a brief phase of increasing depression with a minimum at 10–20 msec and thereafter gradual recovery to normal at about 100 msec. Any early phase of lessening depression or frank facilitation has been attributed to a failure of the antidromic conditioning volley to enter the motoneurone cell body (see later). In considering Lorente de Nó's early findings regarding the refractory period of oculomotoneurone synapses (Lorente de Nó, 1935), the possibility of multiple innervation of individual internal rectus muscle fibre has to be considered (Hunt & Kuffler, 1954). A maximum muscle action potential need not necessarily indicate a maximal discharge of the oculomotoneurone pool.

G. H. Bishop & O'Leary (1940), Marshall & Talbot (1940, 1941) and Talbot & Marshall (1941) made early observations on the recovery cycle of lateral geniculate neurones. Under the conditions in which these early experiments were done no account was taken of presynaptic excitability change and post-discharge supernormality was not distinguished from facilitation by summation of subliminal excitation. Later Marshall & Talbot (1942) and Marshall (1949) attempted to offset presynaptic excitability changes by adjusting the strength of the testing shock. Marshall (1949) concluded that, for shock intervals up to 10–12 msec, refractoriness and supernormality occur and that the succeeding phase of depression lasted up to 5 sec. Marshall's supernormal phase lasted rather longer than we have found, presumably because it includes the summation of subliminal excitation as well as true post-discharge supernormality. The main (late) phase of supernormality reported in this paper

lasted for shock intervals up to 11·3 msec (mean 6·7) with a peak at a mean shock interval of 4·7 msec. The succeeding depression was maximal at a shock interval of 19 msec and thereafter recovery proceeded relatively rapidly up to 87 % of normal at 220 msec and then very much more slowly until normality was achieved at 2 sec. Recently Vastola (1959) studied the recovery cycle of geniculate neurones, using both antidromic conditioning and testing volleys. The antidromic cell body spike recovers nearly to its control height by 6 msec and then enters a prolonged phase of depression. Vastola's Fig. 7 shows the latter to be maximal at 20–30 msec and recovery to be complete between 100 and 150 msec.

The recovery of transmission in a synaptic system will depend, in a complex way, upon the diverse recovery processes in its individual parts, only one of which is the cell body of the post-synaptic neurone. One of the difficulties in determining the pattern of recovery in the individual parts of the neurone is that of assigning electrical activity to histological structure. Unfortunately, we still know relatively little about the properties of dendrites. The many factors which determine the nature of the recovery cycle of the synaptic system may be briefly reviewed.

The recovery cycle of the cell body will depend upon the way in which excitability is tested, i.e. whether by an orthodromic or antidromic volley or by direct stimulation with implanted electrodes. The recovery of the synapse as a functional whole, on the other hand, can only be tested by conditioning and testing volleys both of which are orthodromic and which also traverse the same presynaptic pathway (homosynaptic testing). The use of an orthodromic volley in this way is limited by the recovery processes in the presynaptic pathway and tests excitability changes in the post-synaptic neurone only within the limits fixed by the number and arrangement of the active presynaptic endings. By making the conditioning volley subliminal for a post-synaptic discharge it is possible to study subliminal fringe facilitation without the complication of post-discharge recovery processes (Lloyd, 1946). It is practically impossible to do this in the lateral geniculate nucleus under normal circumstances (Bishop & Evans, 1956; Bishop, Burke & Davis, 1958). In order to study post-discharge recovery processes to the exclusion of subliminal fringe facilitation it is necessary to make the conditioning volley supramaximal for all the neurones within the subliminal fringe of the test volley. While this situation can probably be achieved in the lateral geniculate preparation it is apparently not possible in the case of the motoneurone pools in the spinal cord. In the latter situation also the use of a large conditioning shock introduces the additional complication of interneurone activity. Interneurone activity leading to prolonged synaptic bombardment also makes the flexor reflex pathways much less satisfactory for studying the recovery cycle of motoneurone synapses than the monosynaptic

pathway. For these reasons few systematic studies have been made of the recovery of excitability of motoneurones following orthodromic activation (Lorente de Nó, 1935; Bernhard, 1947; Brooks, Downman & Eccles, 1950*b*; Eccles & Rall, 1951) and in particular relatively little information is available about the early part of the recovery cycle of motoneurone synapses in the first few milliseconds, when refractoriness is subsiding and the later stages of recovery are commencing. In so far as it is possible to exclude subliminal fringe facilitation, the orthodromic recovery cycle of motoneurones resembles the antidromic recovery cycle described above fairly closely except in two respects, namely (1) almost complete suppression of reflex discharge during the first 30–60 msec (Brooks *et al.* 1950*b*; Eccles & Rall, 1951), and (2) a greatly prolonged depression that may not approach normality for several seconds (Eccles & Rall, 1951; Jefferson & Schlapp, 1953; Lloyd & Wilson, 1957). The prolonged depression at intervals beyond 100–200 msec is, therefore, probably presynaptic in origin. A comparison of the antidromic recovery cycle of lateral geniculate neurones (Vastola, 1959) with the orthodromic cycle that we have reported here shows that the late prolonged phase of depression is presynaptic in origin.

The recovery of transmission at the synapse may also be tested by applying the testing orthodromic volley in different afferent fibres from the conditioning volley (heterosynaptic testing). This method, which is not practicable in the geniculate preparation, tests recovery of excitability in the post-synaptic neurone without the limitation imposed by recovery in the afferent pathway, but unfortunately subliminal fringe facilitation effects again obscure post-discharge recovery processes. Even with test intervals longer than the survival time of the synaptic facilitation process the recovery cycle may still be complicated by the prolonged depression that may occur, even when the conditioning orthodromic volley is reflexly subliminal (Brooks *et al.* 1950*b*).

Changes in the quantity of transmitter substance released during recovery may be a factor, though relatively little information is available in this regard. Apparently there is little change following a single orthodromic volley and post-activation potentiation of release such as occurs at the frog neuromuscular junction (Hutter, 1952) does not occur at either the motoneurone synapses (Brooks *et al.* 1950*b*; Brock, Coombs & Eccles, 1952) or geniculate synapses (Bishop *et al.* 1958). Marked changes probably occur, however, following repetitive stimulation (cf. Bishop *et al.* 1959). Another factor is the possible persistence of the excitatory action of the transmitter substance either because of the persistence of the transmitter substance itself (cf. Eccles, Fatt & Koketsu, 1954), or, despite the removal of the transmitter substance, the continued presence of a synaptic depolarization (i.e. synaptic potential) on parts of the post-synaptic membrane (e.g. dendrites) not invaded by a conducted impulse. This possibility will be briefly discussed below.

As far as the recovery of the post-synaptic neurone is concerned the use of an antidromic volley for conditioning avoids some of the difficulties associated with an orthodromic volley, but unfortunately introduces additional complications (Eccles, 1955). Antidromic impulses may not invade the cell body, the block being presumed to occur at either of two sites, namely, at the junction of the axon with the initial segment, or at that of the latter with the cell body itself (Brock, Coombs & Eccles, 1953; Frank & Fuortes, 1955; Fuortes, Frank & Becker, 1957). By antidromic testing the absolutely refractory period of the initial segment of the motoneurone is found to be 1·25–1·45 msec or 0·9–1·2 msec, depending upon whether or not the conditioning impulse invaded the cell body. If the cell body discharges it lengthens the refractory period of the initial segment (Brock *et al.* 1953; Fuortes *et al.* 1957). The absolutely refractory period of the motoneurone cell body ranges from 2·5 to 50 msec.

Studies involving the antidromic activation of motor axons have also brought to light the importance of the axon collateral-Renshaw cell mechanism in determining the excitability of the cell and hence the nature of the recovery cycle following activation whether by antidromic or orthodromic volleys. Both antidromic (and therefore orthodromic) inhibition and facilitation may occur (Renshaw, 1941). By recording intracellularly from the motoneurone Eccles *et al.* (1954) have related antidromic inhibition to the discharge of interneurones (Renshaw cells), activated by motor-axon collaterals and, in turn, discharging on to motoneurones. The latency of the inhibitory effect at the motoneurone, judged by the onset of hyperpolarization, is about 1·2 msec after the arrival of the impulses at the region of branching of the collateral from the main motor axon. After orthodromic activation it would follow the initial segment discharge with very little added latency. Antidromic inhibition reaches a maximum 3–10 msec later and has a total duration of about 40–50 msec. Brooks & Wilson (1959) have shown the marked contribution that recurrent inhibition makes to the depression in the motoneurone recovery cycle.

Antidromic or recurrent facilitation has not received much attention, but recently Wilson (1959) has shown that the latency of the facilitatory effect seems to be longer than that of antidromic inhibition by approximately 1 msec, suggesting the presence of at least one more synaptic delay. A related phenomenon, which at present lacks a satisfactory explanation, is the recurrent efferent discharge that may occur in a few motor axons following antidromic impulses in the same axons (Renshaw, 1941). When allowance is made for conduction time, the departure of the recurrent discharge follows about 0·9 msec after the arrival of the antidromic impulse at the motoneurone. It is evident that immediately after antidromic activation some motoneurones pass through a state of increased excitability which is, however, rapidly superseded by the characteristic prolonged phase of depression. A similar

recurrent discharge has been observed in the lateral geniculate nucleus (Bishop, Burke & Davis—unpublished observations).

O'Leary (1940) has described recurrent collaterals from axons of some of the largest cells in the lateral geniculate nucleus but makes note of their relative scarcity. Recently Vastola (1959) considered that the available evidence was against the possibility that recurrent inhibition of the Renshaw-cell type by means of collaterals from the axons of principal cells occurred in the lateral geniculate nucleus. A mechanism of this kind has, however, been postulated as the basis of the repetitive firing that is a common feature of the geniculate discharge (Bishop, Jeremy & Lance, 1953).

Of the greatest importance in determining the recovery cycle of the synapse are the after-potentials of the cell body and to a minor extent the after-potentials of the initial segment and post-synaptic axon. A full discussion of the after-potentials of geniculate neurones in relation to the recovery cycle of the synapses will be reserved for a later paper (cf. Vastola, 1957, 1959). Following either orthodromic or antidromic activation of geniculate neurones the cell body spike is followed by a brief after-negativity which at about 7 msec reverses to a long-lasting deep after-positivity having a duration of 50–120 msec. There is good agreement between the time courses of these after-potentials and the phases of supernormality and the early part of the phase of subnormality described in this paper.

Both negative and positive after-potentials occur in the motoneurone cell body following the spike discharge. Very little attention has been paid to the negative after-potential but measurement of published intracellular records indicates that it only occasionally has a post-spike duration of more than about 2 msec. It rapidly decays to a large positive after-potential that reaches a maximum after 10–15 msec and has a duration of about 100 msec (Brock *et al.* 1952). The recovery cycle of motoneurones described above diverges widely from the curve that is to be expected from the after-potentials, since there is no phase of supernormality to correspond with the negative after-potential. There, is, however, a phase of relative supernormality between refractoriness and subnormality that is attributable to the negative after-potential (Brooks *et al.* 1950*a*, *b*). By intracellular recording Brock *et al.* (1953) demonstrated re-invasion of a cell body by an antidromic impulse during a brief interval (3–6 msec) following refractoriness and before the onset of depression. The negative after-potential in this instance did not reverse to positivity until about 6 msec. Fuortes *et al.* (1957) have confirmed this by direct stimulation of the motoneurone cell body with an intracellular electrode. They found that excitability approached resting threshold with a brief peak between 2–3 msec after the commencement of an antidromic cell body spike. The absence of a phase of supernormality in the recovery cycle of motoneurone synapses probably has a complex origin—prolonged relative

refractoriness, brief negative after-potential, recurrent Renshaw inhibition and weak presynaptic drive. Relative refractoriness may continue for up to 9 msec (Brock *et al.* 1953).

Supernormality in the recovery cycle of lateral geniculate synapses is probably the result of a brief relatively refractory period, a more pronounced negative after-potential, very strong presynaptic drive and possibly the absence of Renshaw-type recurrent inhibition. An additional explanation seems to be required to account for the early phase of supernormality at stimulus intervals between 0·95 and 1·7 msec, and two possibilities may be put forward. (1) There is much evidence (cf. Edwards & Ottoson, 1958) which indicates that the post-synaptic spike is generated in the initial segment. It is possible that the cell body spike may persist for a brief interval after the spike in the initial segment sufficient to provide a powerful catelectrotonic effect upon the latter. Brooks *et al.* (1950*a*) have put forward a similar explanation to account for the recurrent discharge in motor axons. (2) Freygang (1958) and similar unpublished observations from this laboratory provide evidence which suggests that, while most of the post-synaptic membrane may be excited synaptically to produce a post-synaptic potential, the dendritic membrane and possibly also part of the cell body membrane are not excited electrically and do not produce a propagating spike. The persistence of the post-synaptic potential in these regions would lower the threshold of the remainder of the cell body and of the initial segment. The absence of the earlier phase of supernormality in the antidromic recovery cycle (Vastola, 1959) is in keeping with this suggestion. One difficulty with both explanations given above is the fact that, initially at least, the catelectrotonus would have a depressive effect by lengthening the refractory period of the initial segment (Blair & Erlanger, 1933).

The emphasis in this paper has been on the lateral geniculate nucleus as providing an opportunity for studying the properties of sensory neurones and sensory synapses. The part these properties play in the visual process has been ably discussed by Marshall & Talbot (1942).

SUMMARY

1. By electrical stimulation of the optic nerve the recovery of responsiveness of the synapses in the lateral geniculate nucleus has been studied from the termination of absolute refractoriness up to shock intervals of about 5 sec. The conditioning stimulus was usually maximal for both groups of fibres in the optic nerve and the test response varied between 32 and 62% maximal for the more rapidly conducting group of fibres. Two major difficulties were (i) exclusion of summation of subliminal fringe excitation, and (ii) correction for changes in excitability in the optic nerve at the site of stimulation.

2. The organization of the subliminal fringe was studied. The relationship between the amplitudes of the presynaptic spike (t_1) and the post-synaptic spike (r_1) with increasing shocks applied to the optic nerve, both without and with prior conditioning, show that only a small fringe of subliminally excited neurones is present in the nucleus. The fact that, with conditioning, the amount of the facilitation or depression is independent of the amplitude of the r_1 spike over a wide range of afferent bombardments indicates that the subliminal fringe associated with a smaller presynaptic volley is completely discharged by a larger independent volley. From using maximal conditioning and testing shocks it was concluded that a maximal optic nerve volley does not normally have associated with it a fringe of subliminally excited neurones. Analysis of the random fluctuations in the excitability of geniculate neurones and of the phenomenon of post-tetanic potentiation provides evidence in keeping with this description of the organization of the subliminal fringe.

3. In order to provide the recovering synapses with the equivalent of a constant afferent test volley, corrections were applied to offset changes in excitability of the optic nerve at the site of stimulation by reference to the graph relating the amplitudes of the unconditioned presynaptic and post-synaptic spikes taken over a sufficiently wide range of afferent stimulation. The degree of facilitation or depression of the post-synaptic spike during recovery from discharge can be expressed in a way that is independent of the amplitude of the presynaptic spike.

4. The geniculate neurones respond normally as soon as conduction becomes possible in presynaptic fibres. At shock intervals between 0·95 and 1·65 msec, while the optic nerve axons are recovering from refractoriness the geniculate neurones pass through a brief phase of relative supernormality (114%). When the optic tract axons have regained normal responsiveness (1·7 msec) the geniculate neurones may be slightly subnormal.

5. The early phase of relative supernormality is followed by a late phase of true supernormality with a peak (113%) at a mean shock interval of 4·7 msec and ending at 6·7 msec.

6. The succeeding phase of depression was maximal at a mean shock interval of 19 msec (32%). At this time synaptic transmission through the nucleus was largely blocked. Thereafter recovery proceeded relatively rapidly up to 87% of normal at 220 msec and then very much more slowly until normality was achieved at 2 sec. This two-stage recovery from depression was always present.

7. There may be a further phase of supernormal responsiveness having a peak at about 3·8 sec and ending at about 5 sec.

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FOETAL HEAT PRODUCTION IN THE FOWL

BY C. ROMIJN AND W. LOKHORST

From the Laboratory for Veterinary Physiology, State University, Utrecht, Holland

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The embryonic heat production in homoeothermic animals, particularly mammals, is insufficiently known. On account of technical difficulties it is nearly impossible to estimate the metabolic activity of the mammalian foetus by direct calorimetry and all available data have been obtained by determining the respiratory metabolism. Brody (1938) calculated the embryonic heat production by comparing the total heat production of pregnant animals (rat, cow, horse, sheep and pig) with that of the non-pregnant, and the same technique has been used by Murlin (1908) in the dog, by Pommerenke, Haney & Meek (1930) in the rabbit and by Carpenter & Murlin (1911), Sandiford, Wheeler & Boothby (1931) and Root & Root (1923) in man.

In more recent experiments, such as the work of Barcroft (1946), the respiratory activity of the foetal lamb has been determined by gas analysis of the umbilical blood. The investigations of Barron & Meshia (1954) should also be mentioned, since they afford an excellent review of the problem at the present time.

In birds, the foetal heat production may be determined by putting the incubated egg into a respiration chamber and measuring the oxygen uptake and carbon dioxide output. Indirect calorimetry of the fowl's embryo has been carried out by several investigators; the older work has been extensively reviewed by Needham (1931). With a modern technique Romijn & Lokhorst (1951) determined the respiratory metabolism of the avian embryo continuously over the whole period of incubation; the recent literature on the subject was summarized by these authors.

Direct calorimetry was carried out by the Scandinavian investigators Bohr & Hasselbalch (1903), with incubated eggs, during a limited period of incubation, unfortunately not in combination with respirometry; and Needham (1931), discussing the problem of energy sources during embryonic development, wrote: 'Nothing would be more welcome than a redetermination of the heat-production curve of the chick embryo'.

Barott (1937), investigating the influence of several factors on the hatchability of hen's eggs, was the first who determined the heat

elimination of incubated eggs with a respiration calorimeter (closed system) over nearly the whole period of incubation. On account of the relatively low sensitivity of his apparatus, however, his results underline the difficulty in getting reliable figures on the metabolic activity during the first days of incubation. Moreover, the use of not less than about 90 eggs per experiment introduced the possibility of getting wrong results owing to embryonic death in one or more eggs during the experiment. The curious fact should be mentioned that Barott did not compare the figures on heat production, obtained by indirect calorimetry, with those obtained by direct measurement.

It was the purpose of our investigation to measure the O_2 uptake and CO_2 production as well as the heat elimination of a single incubated egg during the whole incubation period, without any interruption. The advantage of using one egg lies in the possibility of comparing the heat production measured with that calculated, and of getting more reliable figures on the respiratory and the thermal quotient of the developing embryo.

METHODS

A respiration-calorimeter has been constructed for the determination of embryonic heat production in a single incubated egg during the whole period of incubation. The calorimeter is based on the gradient principle. Gradient calorimeters for investigation of metabolic activity in man have been developed by Benzinger & Kitzinger (1949), and a more simple apparatus adapted for measurements on small domestic animals by Prouty, Barrett & Hardy (1949). Our subject, the developing chicken embryo, needs special precautions with the equipment, as there must be a carefully maintained incubation temperature of the calorimeter vessel and ventilation adequately adapted to the oxygen need on each day of incubation. The calorimeter vessel consists of a thin-walled cylindrical vessel of copper (Fig. 1, *a*) with a total volume of about 150 ml., isolated from a larger, outer copper vessel (*b*) by a layer of down (gradient layer). The calorimeter vessel contains the incubated egg (*E*) and a heating element (*r*) of constantan wire of accurately known resistance. A thermocouple (copper-constantan) th_1 is fitted to the wall of the vessel and a current from a 6 V battery is adjusted at the beginning of the experiment to such a strength that a heat production of about 3700 cal/24 hr is reached, corresponding with the heat production of a full-term chicken embryo. Turning the egg can be performed by turning the whole calorimeter vessel inside the water-bath (Fig. 1), without interfering with the measurements.

The temperature of the water-bath is then adjusted to such a level that the temperature inside the calorimeter vessel corresponds with the incubation temperature of 37·7° C. In our experimental conditions a gradient of 3·3° C between the vessels *a* and *b* was necessary to realize this requirement. The correct temperature of the calorimeter chamber can be checked by switching the thermocouple th_1 against a 'cold junction' (th_0) which is kept at a constant temperature of 37·7° C in another water-bath. The galvanometer (*G*) should maintain its zero position. We used a 'Multiflex' galvanometer of high sensitivity, in which the light beam falls on a photo-electric cell of differential type (*F*) to provide automatic compensation for the increase in embryonic heat production. The photo-electric e.m.f. is amplified by an electronic amplifier (Brown) and brings a servomotor (*S.M.*) into action. The motor is connected to a helipotentiometer (*H*) by which the total resistance in the circuit of the heating element (*r*) can be altered till the decreased heating current balances

out the increased heat production of the embryo. The light spot of the galvanometer then returns to its original zero position and the servo mechanism stops. Embryonic heat dissipation can be calculated at any moment by reading the number of milliamperes in the heating wire, calculating the corresponding heat by Joule's formula and subtracting this figure from the original number of calories at the beginning of the experiment. An additional advantage of automatic compensation lies in the fact that a small decrease in voltage of the battery is compensated automatically and that a linearity between heat dissipation and galvanometer deflexion is not absolutely necessary. An accurate reading of the current strength could be performed by measuring the voltage drop across a fixed resistance in the circuit.

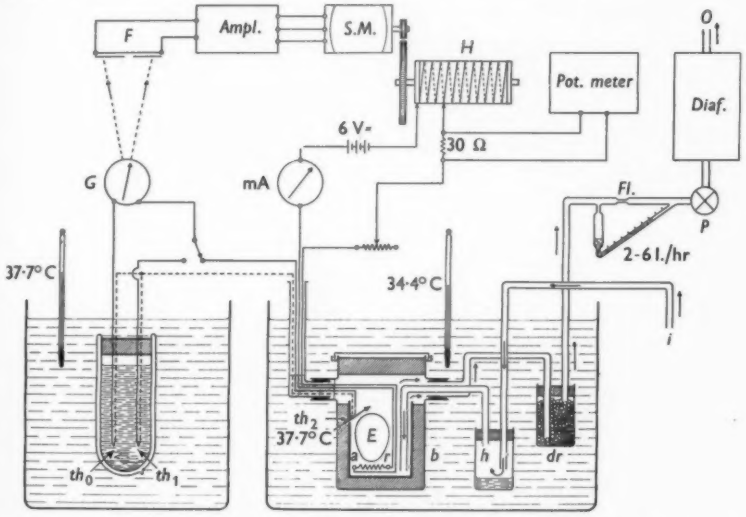

Fig. 1. Respiration calorimeter for measuring heat production and heat elimination of the bird's embryo. *a*, inner wall of calorimeter chamber; *b*, outer wall of calorimeter chamber; *h*, humidifier; *r*, heating element; *th*, thermocouples; *H*, variable resistance (helipotentiometer).

The ventilation of the calorimeter chamber is established by a small pump (*P*), sucking an air stream through the vessel and the flowmeter (*Fl.*). The ventilation was adjusted to 2 l./hr during the first week, 4 l./hr during the second week and 6 l./hr during the third week of incubation. Fresh outdoor air enters at *i*, is warmed up to 34·4° C in the water-bath and brought to a known tension of water vapour in the vessel *h* before entering the respiration chamber. After leaving the calorimeter the air is dried in the vessel *dr*, before entering the gas analyser (*Diaf.*) for continuous recording of the CO_2 surplus and O_2 deficit in comparison with outdoor air. Details of the improved gas analysis and calculation of results in metabolic experiments have been published by Romijn & Lokhorst (1959).

The evaporative heat loss of the egg can be calculated from the increase in weight of the vessel *dr*, after subtracting the amount of water in the incoming air, which is a constant in case of complete saturation at 34·4° C or zero in the case of drying before entry to the respiration vessel. The heat loss of the calorimeter content by convection can be calculated from the amount of ventilating air, its specific heat at 37·7° C, and the assumption that this air will be warmed up from 34·4 to 37·7° C.

Calibration of the apparatus, particularly with respect to direct calorimetry, has been performed by putting into the calorimeter an unfertilized egg with artificial embryo, as has been described by Romijn & Lokhorst (1956). The amount of heat produced by this egg can be adjusted to any desired level and compared with the decrease in calorie elimination of the heating element r in the compensating device. The result of this calibration has been summarized in Table 1, which shows the very close agreement of both figures. The sensitivity of the calorimeter could be calculated as about 3 mV/cal/sec. The galvanometer sensitivity being 1 mm deflexion for 0·6 μV therefore enables the compensation of a calorie elimination as low as 17 calories/24 hr.

TABLE 1. Calibration of the calorimeter with an 'artificial embryo'

cal/24 hr, eliminated by		Decrease in heat elimination of heating element (cal)
Heating element (r)	Artificial embryo	
3714	0	0
3645	82	69
3405	311	309
3244	500	470
3170	550	544
3050	686	664
2800	939	914
2512	1232	1202

RESULTS

Some twenty experiments have been performed with eggs of Blue North Holland hens and of White Leghorns. In nearly all experiments the chicken embryo came to full development in the small incubator and hatching occurred as far as there was room available. The humidity of the ventilating air was adjusted to levels varying over a wide range, and as the tension of water vapour appeared to be of influence on the results further work on the effect of this factor is being carried out. In the present paper the results will be given for experiments with outdoor air without drying or humidifying beforehand. The relative humidity of the air in the respiration calorimeter varied therefore from 10 to 28 %.

As an example, the figures obtained with an egg from a Blue North Holland hen will be given (Table 2 and Fig. 2). From Table 2 and Fig. 2 the conclusion may be drawn that in general the heat dissipation of the developing chicken embryo is in accordance with the heat production calculated from aerobic metabolism even during the first days of incubation. Bohr & Hasselbalch (1903) mention a heat absorption during the first days of incubation; and though Rapkine (1929) gives a possible theoretical interpretation of this phenomenon, Barott (1937) was of the opinion that this heat absorption does not exist but was an artifact. We should emphasize, however, the fact that Barott's technique was not sensitive enough to draw a definite conclusion about this interesting problem.

The high sensitivity of our gas analyser allowed the possibility of estimating accurately the uptake of O_2 during the first days of incubation. In no case were we able to detect an elimination of O_2 by the incubated egg but always a gradually increasing O_2 uptake. Even in unfertilized eggs there is a small, constant O_2 consumption by the egg content. The work of Philips (1941) on the respiration of early chicken embryos should be mentioned. Moreover, most incubating eggs are put into the respiration chamber only after some days of exposure to atmospheric air. The gas tensions in the egg are therefore equal to those in the atmosphere and,

TABLE 2. Heat production of one incubated egg. Egg weight 64 g, temp. 37·7° C, relative humidity 10–28 %

Day of incubation	Heat production of embryo (cal/24 hr)		Evaporative heat loss of the egg (cal/24 hr)	R.Q.	Calorie quotient (cal/g / mg O_2/g)
	Calculated	Measured			
1	16·4	—	403	1·63	—
2	27·2	27·0	362	0·84	—
3	41·7	37·8	362	1·00	3·0
4	62·8	89·0	362	1·00	4·6
5	103	89·0	362	0·94	2·8
6	153	139	357	0·86	3·1
7	215	180	362	0·80	2·8
8	268	276	362	0·92	3·6
9	379	376	362	0·88	3·4
10	574	584	357	0·85	3·5
11	914	888	414	0·81	3·3
12	1314	1296	397	0·74	3·3
13	1874	1726	414	0·69	3·0
14	2326	2196	414	0·71	3·1
15	2685	2510	426	0·69	3·1
16	2812	2683	408	0·69	3·1
17	2671	2716	443	0·67	3·3
18	2830	2873	426	0·69	3·3
19	2550	2698	408	0·73	3·5
20	3477	3124	426	—	—
21	6195	—	—	—	—

depending on the solubility of O_2, N_2 and CO_2 in the egg contents, there will be a greater or less elimination of these gases during and after warming up the egg in the respiration chamber. According to our experience it takes at least 24 hr to bring the temperature of the egg up to the incubating level and the time lapse of 3–4 hr mentioned by Barott (1937) is therefore insufficient to establish an equilibrium between the gas tensions in and outside the egg.

It will be a subject for further investigation to estimate the gas exchange of a freshly laid egg with outdoor air and the gas exchange of the same egg after warming up to incubating temperature. It seems to us that the results mentioned in the literature (Hasselbalch, 1902), in which a possible O_2 production during the first days of incubation is claimed, can be explained by the purely physical exchange of gases between egg content and environment.

A second point of interest is the fact that the evaporative heat loss of the egg is much greater than the heat production of the embryo during the first week of incubation, with the result of an egg temperature somewhat lower than the incubating temperature. Romijn & Lokhorst (1956) found that the temperature of the egg does not rise above that of the

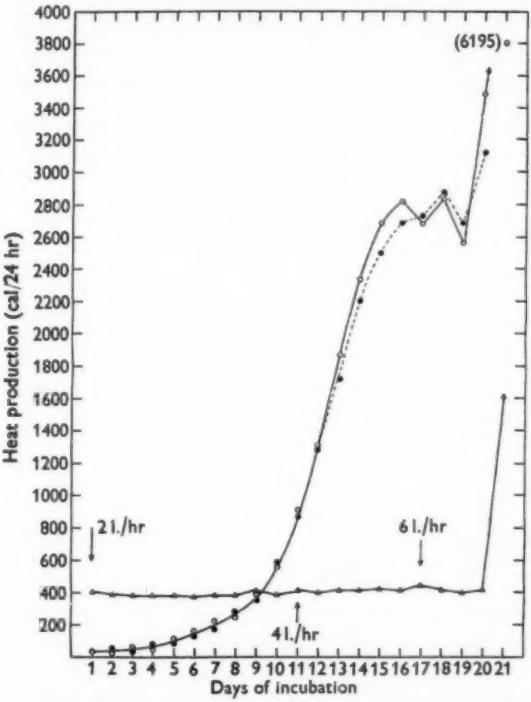

Fig. 2. Heat production of the growing chicken embryo in one egg (64 g, Blue North Holland), estimated by direct (●) and indirect (○) calorimetry; △, evaporative heat loss. Temp. = 37·7° C; relative humidity = 10–28 %.

incubator before the 10th day of incubation, on account of the evaporative heat loss of the egg as a whole. It should be emphasized that during the first days of incubation the total amount of heat given off by evaporation is much greater than the heat production of the embryo: for example, on the 3rd day of incubation the heat production does not exceed one-tenth of the heat loss. The conclusion is therefore justified that a small inaccuracy in the determination of evaporative heat loss is of great influence on the figure obtained for actual heat loss by the embryo. The difficulties encountered by previous investigators in estimating the correct heat

production during early embryonic development may be partly caused by this factor.

Table 2 shows further that increase of ventilation does not coincide with a corresponding increase in evaporation; on the contrary, the evaporation is extremely constant during the whole incubation time and seems therefore nearly completely dependent on the partial pressure of aqueous vapour in the surrounding air, irrespective of its velocity, at least at ventilation rates above 2 l./hr. Keeping the ventilation rate at a constant level of 6 l./hr during the whole period of incubation, we did not find an increase in evaporation corresponding with progress in embryonic development as is mentioned by Barott (1937). Differences in race and breed of the hens may play a part in this respect, owing to modification in the shell structure during the incubation time. With respect to the slope of the curve (Fig. 2) in general we may refer to the paper of Romijn & Lokhorst (1951), in which the metabolic activity of the developing embryo at successive stages is discussed extensively.

The heat production of the incubated egg as measured and that calculated are in very close agreement, at least from the 1st day of incubation up to the 12th. From the 12th day up to the 16th day the uptake of oxygen is slightly greater than the corresponding heat dissipation would suggest. It will be a subject for further investigation to determine whether this difference is a real one. If this be so an explanation of this phenomenon cannot easily be given as it will be very difficult to make a decision about the destination of the extra oxygen. First, there may occur a synthesis of glycogen from the yolk fat, and secondly, there is a possibility of storage of energy during the structural differentiation of the embryo. Moreover, other explanations are possible. The problem of energy retained for organization is an old one and Needham (1931) gives an extensive discussion on this subject, referring to the work of Bohr & Hasselbalch (1903) and particularly to that of Tangl (1903). Fifty years later Barth & Barth (1954) summarized some modern views on the energetics of differentiation: but it should be said frankly that, though the nomenclature has somewhat changed, the problem itself has not yet been solved.

From the figures published by Barott (1937) for the respiratory metabolism of the incubated egg, we calculated the heat production from the O_2 uptake in order to compare this with his figures for direct measurement of heat production (Table 3). Table 3 shows that the amount of heat produced on each day of incubation is greater than the number of calories given off and the discrepancy is on some days, for example, from the 16th to the 18th day, not less than 17 %. The original results of Bohr & Hasselbalch (1903) show a much closer agreement between the amounts of heat observed and the heat calculated from the respiratory metabolism.

The figures published by these authors, however, are very low in comparison with those given by Barott (1937) and those given by us in this publication (Table 2). Bohr & Hasselbalch (1903) found a calorie elimination of 12·16 kcal per egg for the entire incubation period and a calorie production of 12·11 kcal, whereas Barott mentions a heat production of 30·72 kcal and a heat elimination of 25·03 kcal. In the present investigation we find in the egg of Blue North Hollands a heat dissipation of 24·51 kcal and a calculated heat production of 25·28 kcal over the incubation period from the 2nd day up to and including the 20th day. We cannot explain the low figures of Bohr & Hasselbalch and we accept them therefore with reservations.

TABLE 3. Direct and indirect calorimetry of the chicken embryo, calculated from results published by Barott (1937). Relative humidity 24 %, temperature 102° F (39° C).

Day of incubation	Heat calculated from gaseous metabolism (cal/egg/24 hr)	Heat directly measured (cal/egg/24 hr)
5	136	87
6	152	156
7	252	135
8	333	207
9	389	372
10	673	468
11	783	702
12	1119	1002
13	1677	1308
14	2223	1728
15	2350	1956
16	2735	2274
17	2804	2334
18	2892	2445
19	3375	2760
20	3948	3120
21	4884	3975

The respiratory quotient on the successive days of incubation gives an impression about the materials burned in the aerobic metabolism of the embryo. During the first days of incubation the R.Q. is relatively high, according to the CO_2 elimination from the egg-shell and the white. On the 5th day we find a R.Q. of 1·00. It would be erroneous, however, to infer pure glycogen combustion, as on that day the elimination of shell CO_2 is still considerable; a correction for this amount of CO_2 should be made, as has been done by Romijn & Lokhorst (1951). The R.Q. decreases gradually during embryonic development and during the 3rd week of incubation a R.Q. of about 0·70 is maintained (Table 2). Though it may be risky to draw a conclusion about the substances metabolized during embryonic development, it seems likely that mainly fatty acids or fatty acids and proteins play the most important part in aerobic metabolism. In the

literature very erratic figures for the R.Q. can be found, on account of the sometimes primitive method of investigation and the fact that the CO_2 elimination of the egg as such has not been taken into consideration.

The calorific quotient has been calculated from the heat production and the O_2 uptake (Table 2); from the 8th day on the figure for this calorific quotient decreases gradually from 3·6, to 3·0 on the 13th day of incubation,

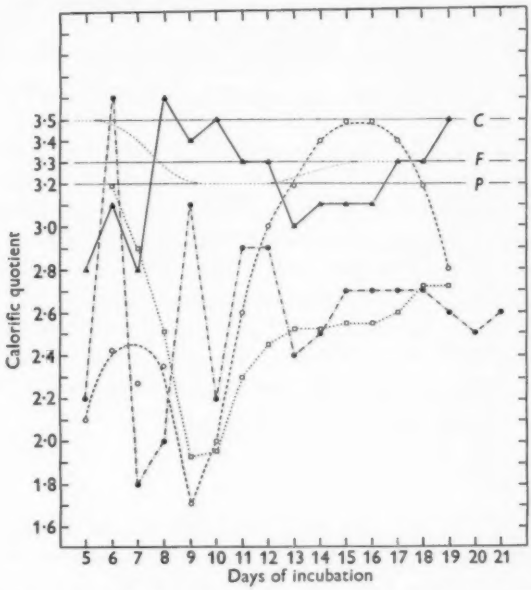

Fig. 3. Calorific quotient of the chicken embryo, calculated from results published by different authors. □, Bohr & Hasselbalch (1903); ○, Hasselbalch (1902); ●, Barott (1937); ▲, this paper.

and after that time there is again an increase up to 3·5. Taking into consideration the very small difference in calorific quotient in case of pure carbohydrate and in case of pure fat combustion it seems somewhat hazardous to draw a conclusion from the calculated figures. Needham (1931), discussing the older work on the respiratory metabolism of the developing chicken embryo, calculated the calorific quotient from Bohr & Hasselbalch's figures for heat dissipation and the figures of some other investigators for the O_2 uptake. After plotting his results in a graph (p. 718) he wrote: 'the dotted line drawn between the horizontal lines shows the course that would theoretically be taken by the calorific quotient, supposing that carbohydrate was first burned, then protein, and finally fat. As can be seen, the experimental curves do more or less follow

that rhythm, but little weight can be attached to such a correspondence in view of the kink on the heat production curve, which almost certainly is responsible for the drop in the calorific quotient at the 9th day.'

Redrawing Needham's graph in a somewhat modified way, we plotted our own figures for the calorific quotient in the same graph, together with those calculated by us from Barott's data on heat elimination and O_2 consumption of an incubated egg at a relative humidity of 24% and a temperature of 102° F (39° C, Fig. 3).

In the original publication of Barott (1937) the calorific quotient has been calculated by him from the CO_2 production of the incubated egg instead of using the figures for O_2 consumption. It seems to us that the latter method of calculation should be preferred to the first method because the CO_2 is partly given off by the shell and the non-living egg content.

The figures obtained from Barott's results are low and remain, one day excepted, below the figure of 3·2 for combustion of protein. Moreover, the factor calorific quotient as such seems to be of questionable importance in the interpretation of results in experimental work on respiratory metabolism; at least Needham (1950), discussing again the metabolism of the chick embryo (p. 591), underlines the importance of the R.Q. for the interpretation of results in metabolic research. The conception 'calorific quotient' is no longer mentioned by this author.

SUMMARY

1. A method has been described for carrying out direct and indirect calorimetry in a single incubated egg during the whole period of incubation.

2. Under normal environmental conditions the results obtained by direct calorimetry agree very closely with the figures calculated from the respiratory metabolism (indirect calorimetry).

3. The respiratory quotient and the calorific quotient have been calculated for each day of development and the results thoroughly discussed in comparison with figures from other authors.

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